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CONTENTS

The effect of root-lesion nematodes on the growth of red clover and alfalfa under greenhouse conditions ... RICHARD A. CHAPMAN 525

Studies of Pratylenchus brachyurus on peanuts ... J. M. GOOD, L. W. BOYLE, AND R. O. HAMMONS 530

Observations on the pathogenicity of biochemical mutants of Pseudomonas tabaci
E. D. GARBER AND HOWARD E. HEGGESTAD 535

Distribution, host range, and origin of lettuce powdery mildew
W. C. SCHNATHORST, R. G. GROGAN, AND ROY BARDIN 538

Influence of moisture content, temperature, and time on the deterioration of stored corn by fungi
SUBHI A. QASEM AND CLYDE M. CHRISTENSEN 544

The effect of pre-inoculation temperature on test plant susceptibility to alfalfa and tobacco mosaic viruses ... J. D. PANZER 550

The control of soaking injury of barley seed ... S. H. F. CHINN AND R. C. RUSSELL 553

Histology of certain oaks infected with the oak wilt fungus
B. ESTHER STRUCKMEYER, J. E. KUNTZ, AND A. J. RIKER 556

Leafspot of marigold, Tagetes erecta, caused by Septoria tageticola n. sp.
WINIT CHANGSRI AND GEORGE F. WEBER 561

Some factors that influence development of dwarf bunt in winter wheat
L. J. TYLER AND N. F. JENSEN 565

Soil fumigation experiments for the control of the soybean cyst nematode, Heterodera glycines
B. Y. ENDO AND J. N. SASSER 571

"Deep plowing" for controlling Verticillium wilt of mint in muck soils ... RALPH J. GREEN, JR. 575

Book Listings ... 577

PHYTOPATHOLOGICAL NOTES:

Host-parasite relationship of the soybean cyst nematode in resistant soybean roots ... J. P. ROSS 578

Germination studies with spores of the dwarf bunt fungus ... WADE G. DEWEY AND L. J. TYLER 579

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THE EFFECT OF ROOT-LESION NEMATODES ON THE GROWTH OF RED CLOVER AND ALFALFA UNDER GREENHOUSE CONDITIONS[1]

Richard A. Chapman

SUMMARY

The growth of red clover and alfalfa in soil infested with a root-lesion nematode population made up primarily of *Pratylenchus penetrans* (Cobb) Filip. & Stek. and *P. coffeae* (Zimmerman) Goodey with some *P. minyus* Sher & Allen was significantly less than that of control plants. Growth was measured by weighing frequent harvests of the plants, and significant effects did not occur until several weeks after cutting was begun. Both species of plants were more severely injured by the nematodes during the summer months than during the winter and spring seasons. Alfalfa, but not red clover, exhibited periods of recovery following periods of significant injury. The nematodes multiplied readily in the roots of both hosts.

Red clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.) have been frequently reported as hosts of root-lesion or meadow nematodes. However, the effects of these nematodes on these plants have received little attention. Hastings and Bosher (5) found that the root-lesion nematode, *Pratylenchus pratensis* (de Man) Filip., reduced the growth of red clover seedlings 64.9 per cent in the absence of fungi and bacteria, and 85.3 per cent when the fungus *Cylindrocarpon radicicola* Wr. was also present.

Good and Blue (2) raised Ladino clover (*Trifolium repens* L.) in methyl-bromide-fumigated and in non-fumigated soil that was infested with *P. brachyurus* (Godfrey) and the fungus *Sclerotinia sclerotiorum* (Lib.) DBy. Cuttings were made at intervals after the plants were 103 days old. After the plants were 197 days old the dry-weight yields of the plants growing in non-fumigated soil were significantly lower than those of plants growing in fumigated soil. The reductions in yield for the 3 cuttings having significant differences, in order of their succession, were 25.9, 65.8, and 87.7 per cent. Examination of roots and soil from the fumigated treatment revealed only traces of both organisms, whereas they developed abundantly in the non-fumigated treatment.

An association between large numbers of *Pratylenchus* and reduced stands of spring-sown alfalfa was reported by the author (1). The present investigation was undertaken to determine the effect of this population of nematodes on the growth of red clover and alfalfa. At the time of the previous report the specific identity of the nematodes was uncertain. A population of the nematodes was maintained in the greenhouse and later thought to be primarily *P. penetrans* (Cobb) Filip. & Stek. It was so considered at the start of this study. After the conclusion of these experiments, however, closer examination revealed that this population was composed primarily of *P. penetrans* and *P. coffeae* (Zimmerman) Goodey, with some *P. minyus* Sher & Allen.[2]

MATERIALS AND METHODS.—Nematodes used as inoculum were obtained from roots of corn (*Zea mays* L.) by the Young (9) incubation technique. The corn had been growing in soil infested with a population of the nematodes that had been maintained in a greenhouse for more than a year. The nematodes were apportioned by diluting suspensions of them with water. The suspensions contained other nematodes, but no stylet-bearing species except *Pratylenchus*. Hereinafter the word "nematode" refers to *Pratylenchus*.

One-half-gallon glazed crocks were filled to within 2 in. of the top with soil that had been heated with steam to 80–85°C for 5 minutes. Fifty-ml suspensions containing known numbers of nematodes were poured over the surface and the crocks were filled with steamed soil. Certified seed of Kenland red clover and Atlantic alfalfa were sown on the surface of the filled crocks and covered with a thin layer of steamed soil. A suspension of a commercial preparation of nodule-producing bacteria was applied when the crocks were first watered. After about 3 weeks the plants were thinned to the desired number per crock by clipping off the shoots of excess plants below the soil line. Control crocks received 50 ml of the suspensions that had been freed of nematodes by gradually heating them to 50°C and immediately removing them from the source of heat.

Growth was measured by weighing plant material obtained by cutting the shoot growth back to a height of 2 in. at intervals during the experiments. Both fresh and dry weights were determined, the latter by drying the material at 40°C until it reached a constant weight.

At the conclusion of the experiments, roots were carefully removed from the crocks and washed, blotted, and weighed. They were then placed in flasks of water on a rotary shaker or hung in a mist chamber (a box filled with fine mist from atomizers and from

[1] Accepted for publication May 8, 1958.
The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director.

[2] I am indebted to Prof. Gerald Thorne of the University of Wisconsin for the identification of the species of *Pratylenchus* in this population.

PHYTOPATHOLOGY for September (48:465-524) was issued October 27, 1958.

TABLE 1.—*Dry weights, in grams, of Kenland red clover growing in soil initially infested with different numbers of root-lesion nematodes*

Harvest No.	Age in days	Root-lesion nematodes added per crock			
		0	500	1180	2700
1	105	1.85	1.68	1.73	1.43
2	132	1.13	1.05	1.00	0.88
3	162	1.23	1.10	1.05	0.83*
4	182	2.00	1.93	1.73	1.63*
5	204	2.55	2.40	1.90*	1.88*
6	214	1.33	1.28	0.90*	1.05
7	230	1.58	1.10*	0.83*	0.68**
8	256	2.63	0.75**	0.83**	1.25**
9	271	0.93	0.20**	0.20**	0.23**
10	292	1.03	0.18**	0.20**	0.18**
11	313	0.38	0.10**	0.13**	0.10**
12	336	0.63	0.15**	0.13**	0.13**
Total		17.27	11.92*	10.63**	10.27**

* Difference from control significant at the 5% level.
** Difference from control significant at the 1% level.

which water containing nematodes drips off roots through funnels into collecting vials). The nematodes were collected and counted for about 2 weeks.

Two experiments were conducted. In Experiment I the numbers of nematodes added per crock in 4 treatments were: 0, 500, 1180, and 2700. There were 4 replicates of each treatment arranged in 4 × 4 Latin squares, 1 for red clover and 1 for alfalfa. The plants were thinned to 20 per crock for each species. The experiment was started on September 20, 1956, and the red clover was harvested 12 times and the alfalfa 13 times (Tables 1 and 2). In Experiment II the numbers of nematodes added per crock in 2 treatments were: 0 and 1750. There were 4 replicates of each treatment for both red clover and alfalfa. The plants were thinned to 5 and 10 per crock for red clover and alfalfa, respectively. This experiment was started on March 12, 1957, and both species were harvested 7 times, at 44, 60, 84, 98, 119, 147, and 181 days of age.

Results of the 2 experiments were similar, and only the data from Experiment I are tabulated here.

RESULTS.—*Red clover.*—The dry weights from the harvests of red clover from Experiment I are presented in Table 1.

In both experiments, although plants growing in soil infested with nematodes produced consistently lower yields than the control plants, significant differences did not begin to appear until some time after cutting was started. In Experiment I this delay was 57 days (2 cuttings), 99 days (4 cuttings), and 125 days (6 cuttings) for the high, medium, and low numbers of nematodes, respectively. The yields from plants in all 3 inoculated treatments were significantly lower than those from the control plants from the seventh through the twelfth harvests. Total yields of the 3 inoculated treatments were significantly lower than those from the control plants.

Growth curves shown in Fig. 1 indicate that the nematodes became gradually more effective in reducing growth from the point when the rate of growth of the control plants began to decrease (214 days, sixth harvest). This decreased rate of growth of the control plants can be attributed to a combination of decreased fertility, effects of frequent cutting, and high temperatures and long days of summer (the seventh harvest was made on May 8, 1957). The tendency toward an increased growth rate shown for the control plants by the upper end of the curve indicates that season may have been a major factor. The last harvest was made on August 22, 1957.

The ranking of the yields of the treatments and the pattern of the appearance of significant differences among the treatments in the different harvests is what would be expected if *Pratylenchus* produces a deleterious effect on the growth of red clover.

The results for the younger plants in Experiment II were similar. Significantly lower yields of the inoculated plants did not appear until 75 days (4 harvests) after cutting was started. Again, the effect of the nematode gradually increased after a rather marked decrease in the rate of growth of the control plants. The change in the rate of growth of these plants was more abrupt, and occurred a month later in the season than that of the plants in Experiment I.

In both experiments the percentages of dry weights (dry wt./fresh wt. × 100) varied somewhat among harvests, but remained fairly constant among treatments until the last 3 harvests in Experiment I and the last harvest in Experiment II. In these harvests the control plants yielded notably higher percentages of dry weights. However, variation within treatments was high, and the differences were not statistically significant.

The data in Table 3 show that, at the conclusion of Experiment I, both the number of plants and the fresh weight of roots were significantly lower in the inoculated treatments. However, these two effects were not proportional. The average root weight per plant was 0.27 g for the control plants, and 0.13, 0.15, and 0.14 g for the plants growing in soil initially

TABLE 2.—*Dry weights, in grams, of Atlantic alfalfa growing in soil initially infested with different numbers of root-lesion nematodes*

Harvest No.	Age in days	Root-lesion nematodes added per crock			
		0	500	1180	2700
1	105	2.15	1.90	2.03	1.88
2	132	1.28	1.18	1.15	0.98
3	162	1.63	1.38	1.23**	1.15**
4	182	1.93	1.58*	1.43**	1.33**
5	204	1.80	1.53	1.15**	1.48
6	214	1.23	1.05	0.75*	0.65*
7	230	2.10	1.08*	0.95**	0.80**
8	256	1.95	1.23**	1.20**	1.08**
9	271	1.25	0.53**	0.45**	0.50**
10	292	1.13	0.55**	0.50**	0.58**
11	313	0.88	0.28**	0.38**	0.25**
12	336	1.38	0.65**	0.68**	0.73**
13	362	1.05	0.83	0.85	0.85
Total		19.76	13.77**	12.75**	12.26**

* Difference from control significant at the 5% level.
** Difference from control significant at the 1% level.

infested with 500, 1180, and 2700 nematodes per crock, respectively. The number of nematodes per g of roots from the individual crocks varied considerably, and differences among the inoculated treatments were not significant. The populations had reached essentially the same level during the experiments.

The numbers of nematodes per g of roots shown in Table 3 are not maximal. Collecting was stopped before the roots were exhausted of nematodes because it was apparent that numbers great enough to indicate multiplication had been obtained.

The results from Experiment II were similar except that the number of surviving plants in the nematode-infested soil was not significantly lower than that of the control. The average weights of roots per plant were 1.07 g for the control and 0.55 g for the inoculated treatment.

The injury caused by the nematode to the roots of red clover seems to be a more important factor in the results of these experiments than the reduction in the number of plants. The average yield of shoot growth per plant can be determined with certainty only with data from the final harvest because stand counts were not made during the experiments. The average dry-weight yields per plant at the last harvest were 0.038, 0.019, 0.027, and 0.026 g for the treatments receiving 0, 500, 1180, and 2700 nematodes, respectively, in Experiment I, and 0.25 and 0.11 g for the control and inoculated plants, respectively, in Experiment II.

TABLE 3.—*Number of surviving plants, fresh weight of roots per crock, and number of root-lesion nematodes per gram of roots of Kenland red clover and Atlantic alfalfa growing in soil initially infested with different numbers of root-lesion nematodes.*

Nematodes added per crock	Plants per crock	Plants surviving per crock	Root wt. per crock (g)	Nematodes per g roots
Red clover				
0	20	16.8	4.55	0
500	20	7.8*	1.03**	18,097
1180	20	4.8**	0.73**	30,849
2700	20	5.0**	0.73**	46,342
Alfalfa				
0	20	19.0	11.99	0
500	20	11.5	7.17*	4,391
1180	20	13.3	6.97*	2,772
2700	20	12.5	6.61*	2,150

* Difference from control significant at the 5% level.
** Difference from control significant at the 1% level.

Alfalfa.—The dry weights of the harvests of alfalfa are presented in Table 2. The results with alfalfa are similar to those with red clover, with 2 notable differences. With alfalfa the deleterious effect of the nematodes appeared sooner after cutting was started

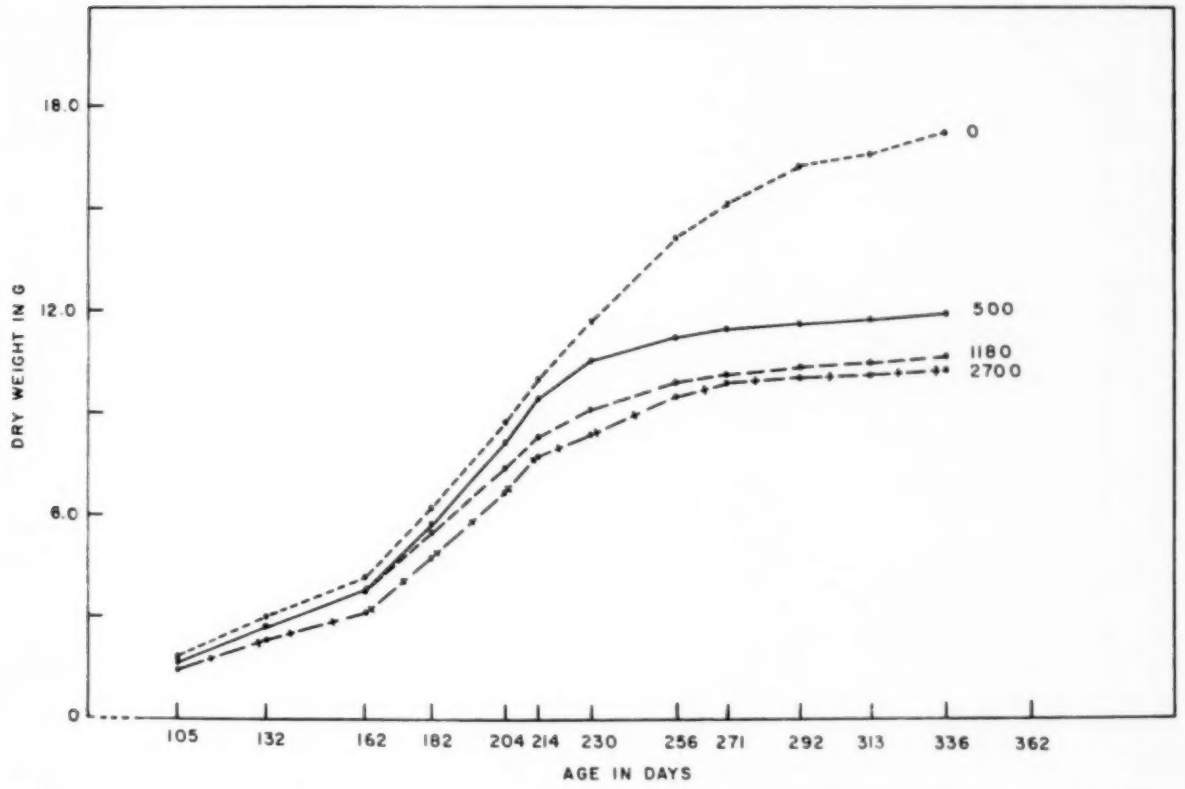

FIG. 1.—Cumulative dry weights of Kenland red clover growing in soil initially infested with different numbers of root-lesion nematodes. The figures at the ends of the curves denote the number of nematodes added per crock.

than with red clover, and there were periods of recovery with alfalfa that were not apparent with red clover. The delay after cutting was started in the appearance of significant differences was 57 days (2 cuttings) for the medium and high concentrations, and 77 days (3 cuttings) for the low concentration of nematodes. Total yields of plants in all 3 inoculated treatments were significantly lower than the total yield of control plants.

Periods of recovery are shown by the changes in significance of differences in yield at the fifth and thirteenth, sixth and thirteenth, and fifth, sixth, and thirteenth harvests from plants in the high, medium, and low concentration of nematodes, respectively. The effect of season is indicated by the fact that these periods of recovery occurred before and after the summer season. The fifth, sixth, and thirteenth harvests were made on April 12, April 22, and September 17, 1957, respectively. As with red clover, the effect of the nematodes was more severe during the summer months.

Growth curves for alfalfa are shown in Fig. 2. Although similar to those for red clover they indicate a more consistent rate of growth for the control plants. The association between a decreased rate of growth of control plants and an increasing effect of the nematode was not as apparent with alfalfa as it was with red clover.

The results for the younger alfalfa plants in Experiment II were more nearly like those for red clover. Periods of recovery were not apparent. Significantly lower yields of inoculated plants appeared 54 days (3 harvests) after cutting was started. Again, this was earlier than with red clover. As in the red-clover Experiment II, the effect of the nematodes increased after a decrease in the rate of growth of the control plants.

The recovery of alfalfa from periods of significant nematode injury is further indicated by calculating the average yield of shoot growth per plant. Again, this can be done with certainty only with data from the last harvest. In Experiment II the average dry-weight yields per plant of the final harvest were 0.17 and 0.067 g for the control and inoculated plants, respectively. However, in Experiment I they were 0.055, 0.072, 0.064, and 0.067 g for the plants receiving 0, 500, 1180, and 2700 nematodes, respectively. In Experiment I the alfalfa was in a period of recovery at the final harvest. Assuming the same number of plants at the next-to-last harvest, the average dry-weight yields for the same order of treatments were 0.073, 0.057, 0.051, and 0.058 g.

As with red clover, the percentages of dry weights in both experiments varied somewhat among harvests, but remained uniform among treatments until the last cutting in Experiment II, when the control plants yielded notably higher dry weights. This result was obtained only in the eleventh cutting in Experiment I.

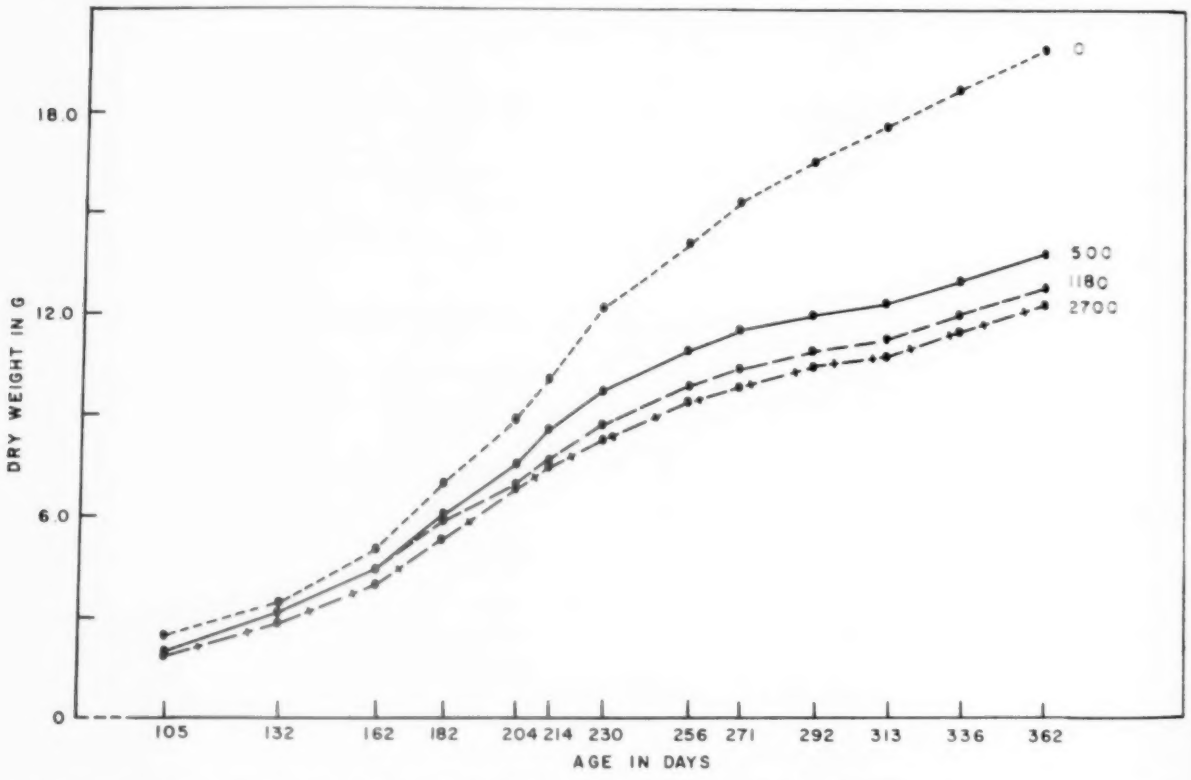

FIG. 2.—Cumulative dry weights of Atlantic alfalfa growing in soil initially infested with different numbers of root-lesion nematodes. The figures at the ends of the curves denote the number of nematodes added per crock.

As with red clover, the differences were not statistically significant.

The number of surviving plants, fresh weight of roots per crock, and number of root-lesion nematodes per g of roots at the conclusion of Experiment I are presented in Table 3. The average root weights per plant were 0.63, 0.62, 0.52, and 0.53 g for, respectively, the control plants and the plants growing in the low, medium, and high concentrations of nematodes.

These weights indicate that, for the differences in root weight per crock to be significantly different, the differences in number of plants per crock should have been significant. Apparently, by the end of the experiment, root weights had become maximal for the conditions of each treatment, regardless of the number of plants. The number of plants per crock varied widely within treatments.

The differences among the number of nematodes per g of roots are not significant, and, as in the red clover experiments, the number of nematodes is not maximal.

For Experiment II the average root weights per plant were 0.98 and 0.57 g for the control and inoculated plants, respectively. These results are similar to those for red clover. The weight of roots per crock was significantly lower from the plants growing in the nematode-infested soil, but there was no significant difference in the number of plants per crock.

Discussion.—The results obtained in these experiments are attributed primarily to the deleterious effects of the root-lesion nematodes on the growth of red clover and alfalfa. However, because of unidentified fungi and bacteria in the inoculant, the results cannot be attributed solely to the nematodes. The controls and the nematode-inoculated treatments probably received different fungal and bacterial populations: the heat treatment was effective in eliminating nematodes from the suspension, but is believed to have been relatively mild so far as its effects on fungi and bacteria are concerned.

For both red clover and alfalfa the effect of the nematodes did not become marked until some time after the cutting of top growth was begun. The yields of the first harvest of red clover in both experiments and alfalfa in Experiment I were slightly lower from plants growing in nematode-infested soil than from the control plants. In Experiment II there was no difference between the yields of alfalfa in the first harvest. A similar delay was obtained with Ladino clover by Good and Blue (2). This delay indicates that these nematodes may not be particularly harmful when plants are under favorable conditions for growth, but that they can become very effective pathogens under adverse conditions for growth. It has been shown repeatedly (3, 4, 6) that frequent and close cutting of the top growth of many species of plants, including red and Ladino clovers and alfalfa, results in reduced growth, reduced carbohydrate content, and other marked physiological changes of the roots. The over-all effect on the plants is reduced growth. Comparing the growth of the control plants in these experiments indicates that red clover is more severely affected than is alfalfa.

The periods of apparent recovery from injury by the alfalfa plants indicate a fundamental difference between the reactions of red clover and alfalfa to these nematodes. If the activity of the nematodes is confined to the smaller, branch roots of the tap roots of alfalfa, it might be expected that, after a period of multiplication of the nematodes, the small roots would be so badly damaged that the nematode population would decrease. While the nematode population is low the plants would recover, drawing on stored food reserves in the tap roots and producing new branch roots. As the new branch roots develop, the nematode population would increase until severe root damage occurs again. Whether correlative nematode population changes and changes in the amounts of root damage and plant growth actually occur remains to be determined. Such a cycle might not be expected to occur in red clover, with its more fibrous root system.

The effect of the nematodes was generally more severe during the summer months. Both *P. coffeae* (8) and *P. minyus* (7) have a direct relationship between multiplication rate in the roots of strawberry (*Fragaria chiloensis* Duch.) and tobacco (*Nicotiana tabacum* L.), respectively, and increasing soil temperature. If this relationship is also true for *P. penetrans*, the increased temperatures of the summer months, coupled with the weakened condition of the plants resulting from the frequent cuttings prior to the summer, would result in a favorable situation for demonstrating the pathogenicity of the nematodes.

Because red clover and alfalfa are perennial plants, it would be expected that, during several growing seasons, defoliation by disease, insects, mowing, and grazing and the effects of such factors as drought and winter injury would frequently provide conditions conducive to severe injury by these nematodes. Thus, these nematodes could be important factors in the loss of stand and production of established plants.

Kentucky Agricultural Experiment Station
Lexington, Kentucky

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STUDIES OF PRATYLENCHUS BRACHYURUS ON PEANUTS[1]

J. M. Good,[2] L. W. Boyle,[3] and R. O. Hammons[2]

SUMMARY

Several experiments were conducted to establish host-parasite relationships and pathogenicity of root-lesion nematodes, *Pratylenchus brachyurus* (Godfrey, 1929) Goodey, 1951, on peanuts and to define the role of this parasite in the peg rot disease of peanuts. Root-lesion nematodes were found in the roots, pegs, and mature shells of peanuts, but were most numerous in the shells, where they colonized in dark-colored necrotic lesions. Infected shells remained a source of inoculum after being cured and stored over winter. Aside from the various degrees of debility shown by infected plants, infection of the pegs was correlated with a peg rot condition resembling the peg rot disease caused by *Sclerotium rolfsii*. Root-lesion nematodes and necrotic peg and shell lesions were reduced in field plots by soil fumigation.

Little is known concerning the pathogenicity and economic importance of plant parasitic nematodes other than root-knot species on peanuts in Georgia. The root-knot nematodes—*Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, and *M. hapla* Chitwood, 1949—have been reported as parasites of considerable importance on peanuts in the major peanut-producing counties of south Georgia, south Alabama, and north Florida (3, 6, 12). Inasmuch as root-lesion nematodes, *Pratylenchus* spp., occur as frequently as root-knot nematodes in these peanut-growing sections, the series of investigations reported in this paper was initiated to determine the pathogenicity of root-lesion nematodes on peanuts, effect of parasitism on peg development, survival of root-lesion nematodes in peanut shells, their relation to peg rot, and the effectiveness of soil fumigation in controlling these parasites on peanuts under field conditions.

The first report of root-lesion nematodes on peanuts in the United States was made by Steiner (8), who illustrated the conspicuous shell lesions on Spanish and Runner peanuts caused by an undescribed species of *Pratylenchus* collected at Holland, Virginia. Later (9) he described the smooth-headed meadow nematode, *P. leiocephalus* Steiner, 1949, on peanuts collected near Fairhope, Alabama. Boyle (1) found large numbers of *P. leiocephalus* in dark-colored pock marks on Spanish peanuts in Georgia. He also observed that these necrotic lesions were not so conspicuous on the thicker-shelled Virginia Bunch peanuts. In South Carolina, Graham (5) found *P. leiocephalus* on peanuts in the greenhouse and field but did not find *P. zeae* Graham, 1951, which is common on corn in that state. Sher and Allen (10) synonymized *P. leiocephalus* with *P. brachyurus* (Godfrey, 1929) Goodey, 1951, and reported its occurrence in peanut shells and roots from several locations in Georgia. Good, Robertson, and Thompson (4) reported *P. leiocephalus* on peanuts at the North Florida Experiment Station, at Quincy. Thus, in cases where root-lesion nematodes infecting peanuts have been identified as to species in the United States, the species has always been *P. brachyurus*.

Crop rotation studies involving root-lesion nematodes have indicated that peanuts are not a favorable host for their reproduction. In North Carolina, Sasser (7) reported that roots of corn and cotton contained about twice as many root-lesion nematodes as roots of peanuts. Graham (5) found in South Carolina that the number of root-lesion nematodes in peanut roots was low during the entire growing season, and that root-lesion nematode counts were lower in tobacco following peanuts than following either cotton or corn. From two- and three-year rotations in Florida, Good (2) found that peanuts significantly reduced root-lesion nematode populations. The more frequently peanuts were grown in the rotation, the lower was the population of these parasites.

Preliminary observations.—Field observations have indicated that pods infected with root-lesion nematodes occur frequently on the Southern Coastal Plain, and that these might be an effective medium for spread of infestation. Susceptible crops were often severely infected with root-lesion nematodes when growing in spots where peanuts had been

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Cooperative investigation between the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Georgia Agricultural Experiment Station.

[2] Nematologist and Geneticist, respectively, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

[3] Plant Pathologist, Georgia Agricultural Experiment Station, Experiment, Georgia.

stacked or picked. A concentration of shells and roots is usually left in the field at these points. In addition, trial plantings in greenhouse pots indicated that peanut shells, not the seed from infected pods, carried an inoculum of *P. brachyurus* (Fig. 1). On one occasion, large populations of root-lesion nematodes were found in a field where peanuts were not harvested (because of adverse weather conditions), whereas an adjacent area of the same field, harvested on schedule, had no outstanding root-lesion nematode problem.

Following an observation that pegs of peanuts in control plots of a soil fumigation experiment were heavily infected by *P. brachyurus*, an attempt was made to determine the percentage of pods left in the ground at harvest because of a weakening or rotting of the mature pegs, or supporting stalks, which was associated with the heavy infection of root-lesion nematodes. In plots treated with standard nematocides, only 6 per cent of the pods were left in the ground, whereas 19 per cent were left in the ground in the unfumigated control plots. The peg rot, and resulting yield losses from leaving part of the crop in the field at harvest, were very similar to that usually attributed to *Sclerotium rolfsii*.

In 1955 a field of Runner peanuts, heavily infested with root-lesion nematodes, was found with chlorotic areas of stunted plants. This condition could not be attributed to extremes of soil moisture, low soil fertility, or any known disease of peanuts. Roots and shells from chlorotic plants harbored more parasites than healthy plants, and, surprisingly, the shells contained about 6 times as many root-lesion nematodes as did the roots.

These observations, made over several years, served as a background for the several experiments reported herein.

GENERAL METHODS.—Unless otherwise stated, root-

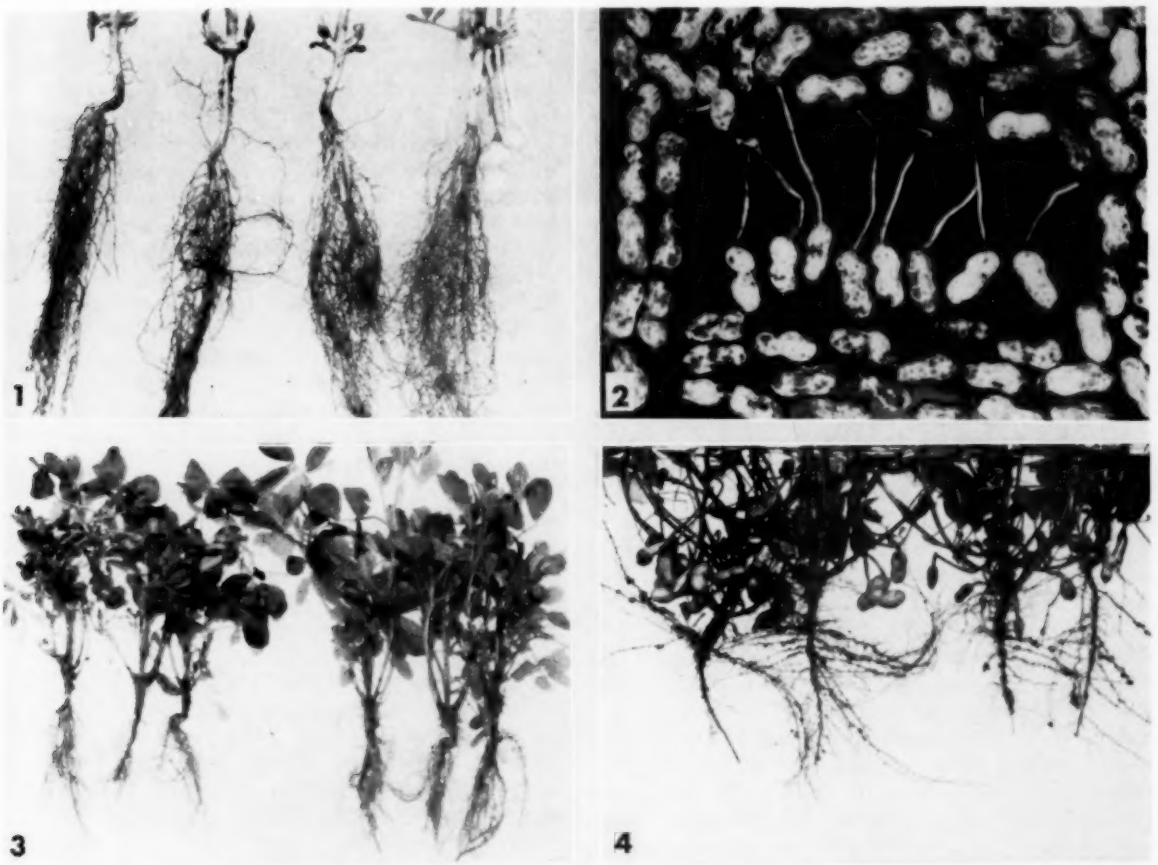

FIG. 1–4. FIG. 1. Carry-over of *Pratylenchus brachyurus* to uninfected soil from infected peanut shells. Three plants on left were infected from nematode inoculum carried in pod of unshelled seed. Plant on right grown from seed removed from infected shell before planting.—FIG. 2. Necrotic peg and pod lesions on Spanish peanuts caused by *P. brachyurus*.—FIG. 3. Effect of root-lesion-nematode-infected peanut shells left over winter in the soil on succeeding planting of Runner peanuts. Three plants on left, grown in soil containing shells infected with root-lesion nematodes, had a large number of parasites in roots. Three plants on right were controls, free of parasites.—FIG. 4. Runner peanut injury from root-lesion nematodes that survived in cured shells stored over winter. Two control plants on left were grown in soil that did not contain infected shells. Two plants on right were grown in soil to which had been added 15 dried shells infected with root-lesion nematodes.

lesion nematode counts were made on 5-g tissue samples, which were processed according to the method of Taylor and Loegering (11). Samples were fragmented in a Waring Blendor for 30 seconds and washed directly into Baermann funnels for nematode extraction. After 48 hours the nematodes were drawn from the funnels and counted.

Methyl-bromide-fumigated soil, a Tifton sandy loam to which was added 4-12-12 fertilizer at a rate equivalent to 350 lb. per acre, was used in all pot experiments. Field plots received 500 lb. per acre of 4-12-12 fertilizer before planting, and an equal amount of gypsum midway in the season. Test plantings included only Spanish and Runner peanuts because previous experience indicated that nematode lesions were less conspicuous on Virginia-type peanuts than on thinner-shelled types.

Occurrence of root-lesion nematodes in shells, roots, and pegs of peanuts.—*Materials and methods.*—During the summer of 1956 a field of Spanish peanuts (not referred to before in this paper) heavily infected with *P. brachyurus* was selected for a study of lesion nematode host-parasite relationships. Several hundred plants were dug and examined to determine the degree of infestation. For root-lesion nematode counts, 10 5-g samples of roots, shells, and seed from infected pods were taken at random from plants collected in the field. Additional observations were made on the occurrence of these nematodes in the elongating pegs, mature pegs, and pods by dissecting the tissue under a microscope. A few shells were preserved in 5 per cent formalin, and later stained with iron alum hematoxylin and counter-stained with safrinin-0, sectioned at 20 μ, and examined for nematodes.

Results.—Large populations of root-lesion nematodes were found in elongating pegs, mature pegs, and pods. No nematodes were found in seed taken from infected shells. Before harvest, large numbers of root-lesion nematodes were found in the tips of young pegs that did not appear to be developing normally. The infected tips of young pods were often decayed and somewhat distorted, but the basal portion appeared normal. Occasionally, colonies of parasites were found in lesions at the junction of the mature pod and peg, resulting in a weakening of the pod attachment point. In addition the peg, or supporting stalk, was frequently attacked at one or more points, causing severe necrosis and shriveling of the mature peg where necrotic pockets of nematodes occurred (Fig. 2). Root-lesion nematodes were difficult to detect in pegs early in the growing season, but were easily detected in mature pegs and pods when shell necrosis became conspicuous shortly before harvest. Lesions on the mature shell, or pericarp, were purplish-brown and could be distinguished from soil microbial decomposition by their darker color and distinct boundaries, which did not fade gradually into the healthy surrounding tissue, as with microbial decomposition. Adults, larvae, and eggs of root-lesion nematodes were found in these lesions; frequently, several hundred adults were dissected from a single lesion. Microscopic examination of fixed, stained, and sectioned pieces of infected shell showed that the nematodes were in the tissue between the vascular network of the pericarp. Several nematodes were observed in a position that suggested they were feeding on cells that lay along the vascular tissue.

Actual root-lesion nematode counts on 5-g samples of shell and root tissue gave an average of 91 parasites in root tissue, and 722 in shell tissue. Based on these averages, the root-lesion nematode population in the shell was about 8 times as great as that in the roots.

Survival of root-lesion nematodes in peanut shells under field and storage conditions.—To establish survival of *P. brachyurus* in peanut shells under controlled conditions, infected shells were left in soil over winter or dried and stored over winter. No effort was made to determine the mechanism of survival or the stage of the nematode that survived.

Materials and methods.—Fifteen freshly harvested nematode-infected Runner peanuts were placed in sterile soil in each of 8 2-gal. pots to determine the over-winter survival of root-lesion nematodes in peanut shells left in the soil. An additional 8 pots, not inoculated with infected pods, served as controls. The pots were left out-of-doors during the winter, and, except for an occasional rain, no water was added during the winter. The following spring, 4 inoculated and 4 control pots were planted with Runner peanut seed. Aristogold sweet corn seed was similarly planted in the other inoculated and control pots. Nine weeks later the soil was washed from around the roots, and the root systems of 2 plants from each pot were placed in jars for nematode extraction by the method described by Young (13). Counts were made on the number of root-lesion nematodes that migrated from the roots after 72 hours.

To determine the survival of root-lesion nematodes in stored peanuts, freshly harvested nematode-infected Spanish peanuts were dried in the sun on trays until the moisture content of seed was 10 per cent, as determined by a Steinlite Moisture Tester. After drying, the pods were stored 8 months in open glass jars at room temperature. The moisture content of pods during the greater part of the storage period was probably about 5 per cent because, under heated and well ventilated storage conditions, pod moisture quickly drops to about that percentage. In June, 1956, shells from 15 dried pods were selected at random and mixed in the upper 2 in. of sterile soil in each of 3 2-gal. glazed pots. Three other pots contained only sterile soil. Four Runner peanut seeds were planted in each pot and, after emergence, thinned to 1 plant per pot. The pots were placed in a slathouse for maintenance. At plant maturity, 4 months later, the soil was washed from the roots for root observations and root-lesion nematode extraction

by the method of Taylor and Loegering (11). Pieces of roots and several mature pods from the pots were checked for pathogenic fungi on nutrient agar plates by H. W. Rankin.[4]

Results.—Peanut shells carried an inoculum of root-lesion nematodes when dried and stored over winter and when left in soil over winter. Peanut roots taken from soil inoculated the previous fall with root-lesion-nematode-infected pods had an average of 143 root-lesion nematodes for 2 plants from each pot. Infected plants had smaller leaves and a more restricted root system than uninfected plants (Fig. 3). The roots of sweet corn from soil inoculated with infected pods had an average of 116 root-lesion nematodes for 2 plants. Infected sweet corn plants showed more firing, and were slightly smaller than uninfected plants. The only discernible difference between infected and uninfected root systems was that infected roots were slightly discolored by small, brown lesions caused by root-lesion nematodes. Root-lesion nematodes were not present in roots of either sweet corn or peanut control plants.

Root-lesion nematodes were found in the roots, pegs, and mature shells of all plants grown in pots inoculated with dried nematode-infected pods that had been stored over winter. None were found in the control plants. Roots of infected peanut plants had an average of 487 lesion nematodes per gram of root tissue. Infected plants were stunted; had decayed root systems, runners, and pegs; and had smaller mature fruit than control plants (Fig. 4). Colonies of miscellaneous microorganisms, including Rhizoctonia, developed on the nutrient agar plates inoculated with root and shell tissue from both infested and uninfested pots. *Sclerotium rolfsii* was not isolated from plant tissue in this study, nor were there visible signs of this organism on the roots, pegs, pods, or stems of experimental plants.

SOIL FUMIGATION FOR CONTROL OF ROOT-LESION NEMATODES AND ASSOCIATED PEG ROT OF PEANUTS.—This experiment was conducted to determine whether soil fumigation, either as a preplanting or post-planting treatment, would control root-lesion nematode infection and reduce pod losses at harvest due to weakness of infected pegs.

Materials and methods.—The experiment consisted of 2 adjacent tests of the same design, with preplanting row fumigants in one and post-planting treatments in the other. Plots were located on a Tifton sandy loam with a history of root-lesion nematode infestation. Each test, a randomized block, contained 6 replications of 4 32-in. rows 25 ft. long per treatment. Preplanting soil fumigants were applied on April 13, 1956, and consisted of D-D (by weight, 50% 1,3-dichloropropene and 50% 1,2-dichloropropane) and Telone (by weight, 95% 1,3-dichloropropene). Both chemicals were applied at the rate of 11.2 gal. per acre in 32-in. rows by tractor-drawn equipment of the gravity-flow type. For the post-planting applications of nematocides, 2 formulations of Nemagon (1,2-dibromo-3-chloropropane) were used: by weight, 70.3% emulsible liquid and 10% technical material on Attaclay granules. The emulsible liquid formulation was applied at rates of 0.9 and 1.8 gal. per acre of technical material, and the 10% granular material was applied at a rate equivalent to 0.9 gal. per acre of technical material. These applications were made at a depth of 3 in., with an equal dosage at 3 in. to each side of the row. Liquid formulations were injected with a handgun at 12-in. spacings along the rows. The granules were spread evenly in furrows that were opened and closed with a garden plow. Post-planting applications were made when plants were beginning to bloom.

All plots were planted with Southeastern Runner peanut seed 10 days after the preplanting fumigants were applied. One hundred and forty days later the plants were dug with a mechanical digger-shaker, and immediately examined for shell lesions. Each plot was rated for prevalence of shell lesions by an index system of 0–4 (0, none; 1, trace; 2, moderate; 3, heavy; and 4, almost completely covered). One quart of pods was taken at random from the vines of each plot; 5-g shell samples were later taken from these field samples for lesion nematode counts.

Yields were recorded in pounds of pods per plot, and the percentage of pods left in the ground at harvest was estimated by recovering the pods left in the soil by sifting the soil from 20 row-feet of each plot over ½-in. hardware cloth. The weight of recovered pods was adjusted to 100 linear feet of row per plot, which figure was used in estimating the percentage of the total crop left in the ground at harvest.

Results.—Preplanting row fumigation with D-D and Telone significantly reduced the number of root-lesion nematodes and effectively controlled these parasites in peanut shells (Table 1); the incidence of necrotic lesions on peanut shells was significantly reduced by both treatments. Peanuts grown in fumigated plots were practically free of shell discoloration, whereas shell discoloration was present in the unfumigated plots.

TABLE 1.—*Effect of preplanting row fumigation on number of root-lesion nematodes recovered from shell tissue of Runner peanuts, and on necrotic-lesion index of mature peanut shells*

Treatment	Av. no. nematodes per 5-g of shell tissue	Av. necrotic-shell index[a]
D-D (11.2 gal./A.)	1.8	0.2
Telone (11.2 gal./A.)	6.5	0.4
Unfumigated check	92.5	2.4
LSD .05	44.9	0.7
LSD .01	75.1	1.0

[a] 0, none; 1, trace; 2, moderate; 3, heavy; 4, almost completely covered.

[4] Plant Pathologist, Georgia Coastal Plain Experiment Station, Tifton, Georgia.

No phytotoxicity was observed for the various Nemagon post-planting treatments, and the numbers of root-lesion nematodes in peanut shells were significantly reduced. The emulsible liquid injection rates of 0.9 and 1.8 gal. per acre of technical material appeared to give slightly better control of these parasites than did the granular material, which contained 0.9 gal. per acre of technical 1,2-dibromo-3-chloropropane (Table 2). Plants that received post-planting applications of Nemagon produced pods that were bright and practically free of discoloration. This was not true of pods from plants grown in unfumigated soil.

No significant difference was found for any of the chemicals used in this experiment for yield of pods harvested or for percentage of the crop left in the ground at harvest. The trend was, however, for a larger pod harvest in treated plots because a greater percentage of the crop was lifted at harvest. Peanut yields and incidence of peg rot and root-lesion nematode injury to pegs and pods were smaller than anticipated, but this disparity was probably related to rainfall during the critical period for pod development. Rainfall during August, 1956, totaled 1.79 in., far short of the 34-year average, 5.44 in., for the Tifton area; it was the second-driest August on record. This drought, coupled with an average monthly maximum temperature of 93.2°F, caused frequent wilting of peanuts for several consecutive days at intervals throughout the month, resulting in sharply curtailed yields in the area. Since adequate soil moisture is prerequisite for extensive activity of *S. rolfsii*, incidence of peg rot was reduced by drought. These conditions, no doubt, also resulted in less nematode damage to pegs and pods. The effect of these factors on yield response in fumigated plots and on pod losses, though not directly measurable, was undoubtedly of considerable importance to the outcome of these experiments.

Discussion.—From the foregoing experiments it is fairly well established that root-lesion nematodes are a primary cause of characteristic dark-colored, necrotic lesions on peanut pegs and pods. These experiments also established that the root-lesion nematode, *P. brachyurus*, can be a destructive parasite of peanuts. Although the damage occurs frequently over a wide area in the peanut belt of Georgia, Alabama, and Florida, the economic importance of this pest in the peanut-growing sections of the Southeast has not been established. On heavily infested land, yield losses from root-lesion nematodes can be attributed to destruction of root tissue, injury to young pegs, and failure to harvest the entire crop. Undoubtedly, root injury is important, but peanut yields will ultimately be determined by normal development of pegs; therefore root-lesion-nematode injury to young pegs appears to be particularly important. Yield losses in the form of reduced pod production have been associated with *P. brachyurus* on a number of occasions from experimental tests and field observations.

Peg rot of peanuts is usually attributed to *S. rolfsii*, but this series of experiments indicates that a peg rot condition may also be caused by root-lesion nematodes. The possibility of a disease complex, involving microorganisms and root-lesion nematodes, was not definitely eliminated, but the evidence suggests that root-lesion nematodes are capable of causing a deterioration or weakness of the peg similar in effect to that produced by *S. rolfsii* infection. The parasitic role of root-lesion nematodes in producing peg rot does not minimize the importance of *S. rolfsii* as a pathogen on this crop plant, because peg rot caused by *S. rolfsii* is a distinct disease; however, *S. rolfsii* and *P. brachyurus* are frequently found occurring together as pathogens.

The concept that peanuts are less likely to increase root-lesion nematode populations than are other crop plants, such as cotton, corn, and tobacco, should be modified, since previous studies on population dynamics involving these crop plants were based primarily on nematodes recovered from soil and root samples. When pods and pegs as well as roots are considered, the peanut plant is a favorable host for *P. brachyurus*, because reproduction in shell tissue is 6–8 times as great as in an equal weight of root tissue. However, the agronomic practice of growing peanuts in rotations with other crop plants is basically sound because many infected pods are normally removed from the field at harvest. Peanut shells, therefore, act as a "trap crop," in that large numbers of these parasites can be removed from the field if the crop is harvested properly. Experience has shown that early harvesting will remove more diseased pods from the field than will delayed harvesting. Timely harvesting of peanuts to remove root-lesion nematodes from the field should be considered an important phase of peanut production, especially if peanuts are grown in a rotation system to reduce root-lesion nematode populations.

Peanut shells are processed and used in a number of different ways, but peanut shell mulches around such plants as ornamentals and strawberries may serve as a source of inoculum for these destructive

Table 2.—*Effect of Nemagon post-planting treatment at blooming on number of root-lesion nematodes recovered from shell tissue of Runner peanuts, and on the necrotic-lesion index of mature peanut shells*

Treatment	Av. no. nematodes per 5-g of shell tissue	Av. necrotic-shell index[a]
Emulsible Nemagon		
0.9 gal./A.[b]	0.0	0.1
1.8 gal./A[b]	0.2	0.1
10% Granular Nemagon		
0.9 gal./A.[b]	14.8	0.1
Unfumigated check	492.0	1.3
LSD .05	171.2	0.2
LSD .01	269.4	0.4

[a] 0, none; 1, trace; 2, moderate; 3, heavy; 4, almost completely covered.

[b] technical 1,2-dibromo-3-chloropropane.

parasites. The studies reported here emphasize the need for further work with commercially processed materials to evaluate each with respect to spread of root-lesion nematodes.

In fields where the root-lesion nematode problem becomes serious, the condition can be corrected by soil fumigation. Row fumigation with standard nematocides and row post-planting treatment with 1,2-dibromo-3-chloropropane, applied when the plants begin to bloom, may prove to be effective in preventing lesion nematodes from injuring pegs and building up large populations in shells. Soil fumigation may also be an aid in the production of certain varieties sold for consumer consumption in the shell. This type of trade requires bright shells, and soil fumigation can eliminate at least one cause of shell discoloration, the root-lesion nematode.

USDA Nematology Field Laboratory
Georgia Coastal Plain Experiment Station
Tifton, Georgia

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OBSERVATIONS ON THE PATHOGENICITY OF BIOCHEMICAL MUTANTS OF PSEUDOMONAS TABACI[1]

E. D. Garber and Howard E. Heggestad

SUMMARY

Biochemical mutants of *Pseudomonas tabaci* (Wolf and Foster) Stevens requiring certain amino acids for growth were inoculated into leaves of 20 varieties of *Nicotiana tabacum* L. and 10 other species of *Nicotiana*. The mutants were not pathogenic for most of the species and varieties, and no species or variety was susceptible to all the mutants. The species *N. longiflora* Cav., *N. repanda* Willd. ex Lehm., *N. rustica brasilia* Schrank, and the *N. tabacum* varieties Burley 21, Bel 6-62, and Bel 248C-2D were resistant to all the biochemical mutants as well as to the parental wildfire isolate R-3. Only the mutant requiring leucine was pathogenic for any variety of *N. tabacum*. *N. glutinosa* L. was susceptible to 4 of the 5 mutants, being susceptible to more mutants than any other host.

Biochemical mutants of pathogens have become valuable tools in studying the role of nutrition in the host-parasite relationship (5). Animal and plant bacterial pathogens and plant fungal pathogens have been used, and the results of these investigations have led to the formulation of a nutrition-inhibition hypothesis of pathogenicity (6); that is, two environments in the host directly affect the fate of the invading parasite: a nutritional environment and an inhibitory environment. In studies with one bacterial plant pathogen, different species and varieties were inoculated with biochemical mutants and a pattern of susceptibility and resistance was observed that could be interpreted as an expression of the relationship between the demand for a required amino acid by the parasite and the supply of this nutrient by the host.

The wildfire bacterium, *Pseudomonas tabaci* (Wolf & Foster) Stevens, produces an exotoxin, detectable as a bacteria-free, chlorotic halo surrounding the necrotic focus of infection in the leaf at the site of inoculation (2). Preliminary experiments (8) indicated that this bacterial species would yield biochemical mutants by procedures used for *Escherichia coli* (Migula) Castel. & Chalm. and *Erwinia aroideae*

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Town. This paper presents observations on the resistance or susceptibility of 20 varieties of *Nicotiana tabacum* L. (commercial tobacco) and 10 other species of *Nicotiana* to 5 biochemical mutants of *Ps. tabaci* and to the parental isolate designated R-3.

MATERIALS AND METHODS.—Procedures for inducing, isolating, and characterizing biochemical mutants of *Ps. tabaci* followed procedures reported in detail for *E. aroideae* (9). A different minimal medium (11) was solidified with Noble agar (Difco) whenever a solid medium was required. The complete medium contained either nutrient broth or nutrient agar (Difco) supplemented with 0.5 per cent yeast extract. All mutants were maintained on a medium containing Bacto-agar, glucose, yeast extract, and calcium carbonate. All were incubated at 30°C.

To prepare inocula for the pathogenicity tests, the bacterial growth was washed off the surface of slants of complete medium, using sterile distilled water. The turbid suspensions were centrifuged, washed once, and resuspended in distilled water. The washing separates the bacteria from nutrients present in the medium and the accumulated exotoxin. The latter was removed because dilutions of the exotoxin, even when separated from the bacteria, may produce lesions on plant species that will not support growth of the bacteria (3). The washed bacterial suspensions were held about 4 hours at room temperature prior to the inoculations.

TABLE 1.—*The response of species of Nicotiana and varieties of N. tabacum (commercial tobacco) to inoculation with Pseudomonas tabaci, strain R3*

Host	Chlorotic halo diameters[a] (mm)
Susceptible:	
N. debneyi Domin.	8–10
N. glauca Grah.	2–4
N. glutinosa L.	8
N. nesophila Johnston	4
N. raimondii MacBride	6–10
N. sylvestris Spegaz & Comes	8–14
N. tomentosiformis Goodspeed	8
N. tabacum L.	
Burley 2	6–12
Catterton	4
Coker 139	2–6
Connecticut 49B	4–14
Dixie Shade	4–12
Gaucharo	8–14
Havana 142	2–10
Hicks Broadleaf	4–8
Kentucky 35	4–0
Madole	4–10
Maryland Mammoth	4–8
Samsun	2
Swarr-Hibshmann	6–16
TI 87 (American)	4–6
TI 170 (Daruma)	4–6
Timor	4
Variety 402	6–8
Resistant:	
N. longiflora Cav.	0
N. repanda Willd. ex Lehm.	0
N. rustica brasilia Schrank	0
N. tabacum L.	
Burley 21[b]	0
Bel 248C2D[b]	0
Bel 6-62[b]	0

[a] One inoculation made on each of 3 plants. Scored 12 days after inoculation (Beltsville, Md., June, 1957).

[b] Varieties and breeding lines with resistance to wildfire transferred from *N. longiflora*.

The varieties of *N. tabacum* used in the experiments are representative of widely different agronomic types, both domestic and foreign. The biochemical mutants were tested on 3 9-week-old rapidly growing plants of each species and variety of *Nicotiana* in 6-in. pots. The 2 largest rapidly expanding leaves—at least 6 in. long and at the middle of the plant or higher—were selected for the inoculations. A drop of the bacterial suspension was placed on the upper surface of the leaf, which was inoculated by needle puncture through the drop. As many as 4 separate tests were made on the same leaf by confining each inoculation to the area between 2 of the larger lateral veins on either side of the midrib. The relative pathogenicity of the mutants was determined by measuring the diameter of the chlorotic halo at the site of inoculation 12 days after inoculation. The parental isolate (R3) of *Ps. tabaci* was used as a control, making 1 inoculation on each of the 3 plants of each host inoculated with the different biochemical mutants.

RESULTS.—The species of *Nicotiana* and the varieties of *N. tabacum* differed in their susceptibility to isolate R-3 of *Ps. tabaci*, ranging from highly susceptible to resistant (Table 1). *N. longiflora* Cav., *N. repanda* Willd. ex. Lehm., and *N. rustica brasilia* Schrank were resistant to isolate R-3, with no lesions developing after the inoculations. Included in the series of hosts were 3 varieties or breeding lines of *N. tabacum*—Burley 21, Bel 6-62, and Bel 248C-2D—having wildfire resistance controlled by a single pair of dominant genes transferred from *N. longiflora* (4, 10). As expected, Burley 21, Bel 6-62, and Bel 248C-2D were resistant to R-3 in these tests. The wildfire-susceptible varieties of *N. tabacum* showed about the same range of susceptibility to isolate R-3 as the susceptible *Nicotiana* species (Table 1).

Relatively few susceptible hosts and none of the resistant hosts listed in Table 1 were susceptible to the biochemical mutants (Table 2). In general, the chlorotic halo exhibited by the susceptible host inoculated with a biochemical mutant was smaller than the halo resulting from the inoculation of the same host with the parental strain. *N. glutinosa* L. was susceptible to 4 of the 5 mutants, being susceptible to more mutants than any other *Nicotiana* species or variety of *N. tabacum*. *N. sylvestris* Spegaz. & Comes was susceptible to 3 of the 5 mutants, and it was one of the most susceptible hosts to the parental isolate R-3. Only the mutant requiring leucine was pathogenic for any of the varieties of *N. tabacum*. Relatively large lesions, of about the same size, were

TABLE 2.—*A summary of the response to Nicotiana species and varieties found susceptible to biochemical mutants of Pseudomonas tabaci*[a]

Mutant	Requirements	Host	Chlorotic halo diameters[b] (mm)
32-3-29	arginine	*N. debneyi*	4-6
		N. glutinosa	4-6
		N. sylvestris	4
26-1-8	leucine	*N. debneyi*	6-8
		N. glutinosa	4-6
		N. sylvestris	2-10
		N. tabacum	
		TI 170 (Daruma)	2-6
		Swarr-Hibshmann	4-8
		Havana 142	6
		Catterton	2-4
4-1-22	serine	*N. glutinosa*	4-8
		N. raimondii	4-8
32-4-6	serine or glycine	*N. glutinosa*	4-6
7-4-33	methionine	*N. sylvestris*	4
		N. tomentosiformis	2-4

[a] All species and varieties listed in Table 1 were tested.
[b] For each mutant, 1 inoculation was made on each of the 3 plants that were used to test *Ps. tabaci*, isolate R3, in Table 1. Scored 12 days after inoculation (Beltsville, Md., June, 1957).

produced on varieties Gaucharo and Swarr-Hibshman following the inoculations with R-3, but, of these varieties only Swarr-Hibshman was susceptible to the biochemical mutant requiring leucine. Other tobacco varieties susceptible to the same mutant were Havana 142, Catterton, and the USDA Tobacco Introduction 170 (variety Daruma).

DISCUSSION.—The pattern of resistance and susceptibility of the host species and varieties to the biochemical mutants of *Ps. tabaci* parallels observations in similar experiments using biochemical mutants of *E. aroideae* and species of vegetable and varieties of radish and turnip (5, 9). In those experiments, slices of the fleshy storage organs were inoculated with the biochemical mutants. It is interesting to note that Boone, Kline, and Keitt (1), using biochemical mutants of *Venturia inaequalis* (Cke.) Wint. and several varieties of apple, did not detect a pattern of susceptibility and resistance for their varieties and biochemical mutants.

Although the nature of resistance of certain species and varieties of *Nicotiana* to *Ps. tabaci* is not yet understood, the nutrition-inhibition hypothesis of pathogenicity (6) offers a possible explanation for the pattern of susceptibility and resistance of the species and varieties (known to be susceptible to *Ps. tabaci*) to the biochemical mutants. According to this hypothesis, the unavailability in the host of 1 or more nutrients required by the parasite for proliferation and metabolism results in avirulence; availability of all required nutrients in the host, however, does not necessarily lead to virulence, since the host may also present effective inhibitory mechanisms. Unavailability of nutrients may result either from a low concentration of these nutrients in utilizable form or from the presence of antagonists or inhibitors that interfere with either the uptake or the utilization of the nutrients. Garber and Shaeffer (7) have presented evidence to support the nutrition-inhibition hypothesis.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS
AND
TOBACCO SECTION
CROPS RESEARCH DIVISION, ARS
U. S. DEPARTMENT OF AGRICULTURE
BELTSVILLE, MARYLAND

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DISTRIBUTION, HOST RANGE, AND ORIGIN OF LETTUCE POWDERY MILDEW[1]

W. C. Schnathorst, R. G. Grogan, and Roy Bardin[2]

SUMMARY

Most reports on the occurrence of powdery mildew on cultivated lettuce in the United States before 1951 were found in error. The report of its occurrence in the Salinas Valley in 1940 is valid, but is based on infection of commercial-wild-lettuce hybrid 41858 by the Salinas wild-lettuce strain, rather than by the new cultivated-lettuce mildew. As far as known, powdery mildew presently occurs on cultivated lettuce only in the United States. It appears to have originated in the Salinas Valley, in California. The strain responsible for a 1951 outbreak on cultivated lettuce in California is physiologically different from the Salinas wild-lettuce strain: it is pathogenic to cultivated-lettuce varieties and more sensitive to high temperature. Host range similarities on other hosts, and mixed infections of the 2 strains on wild lettuce in the field, suggest that the cultivated-lettuce mildew strain is a mutant of the endemic Salinas wild-lettuce strain.

Powdery mildew (*Erysiphe cichoracearum* DC. ex Mérat) (11) of cultivated lettuce (*Lactuca sativa* L.) was widespread in the Salinas Valley in 1951 (13). Perithecia were abundant the following year, and Deslandes, Bardin, and Snyder (6) suggested that the disease might become more prevalent. Powdery mildew has occurred each year in the Salinas Valley since 1951, and in some instances has caused serious damage. Symptoms and damage caused by the disease have been described in previous reports (8, 9, 13) and are illustrated in Fig. 1.

This investigation was conducted to determine 1) the distribution of the disease in California, 2) the role of other hosts in its perpetuation, and 3) the region and mode of origin of the pathogen.

OBSERVATIONS AND EXPERIMENTAL RESULTS.—*Reports of occurrence of mildew on cultivated lettuce.*—The first report of powdery mildew on lettuce in the United States, made by Coons (4), accounts for the Michigan reference in Weiss' host index (15). Coons' report was based on collection No. 319 by Dr. W. J. Beal, made in 1890 at the Michigan State College garden. However, a sample from this collection was identified by the writers as *Septoria lactucae* Pass., not *E. cichoracearum*. This identification was verified by Professor Ernst A. Bessey, who further stated that there were no specimens of *E. cichoracearum* on *L. sativa* in the Michigan State University herbarium.

In 1937 Thompson (14) reported that powdery mildew of lettuce occurred in some parts of the west, particularly the Imperial Valley of California, and that mildew was a serious problem in producing lettuce under glass in the eastern United States. However, through personal correspondence with Dr. Thompson, it has since been established that the disease referred to in that report was, in both instances, downy, rather than powdery, mildew.

Blumer (1) cited 2 references on the occurrence of lettuce powdery mildew in Europe, in 1914 and 1931; but only the oidial stage was found. The identification of the fungus as *E. cichoracearum* was therefore based on the asexual stage rather than the sexual stage. Correspondence with Dr. W. C. Moore (through Dr. L. E. Watts, in England) and Dr. H. Bremer, in Germany, established that the disease has not been observed recently in Europe. The European text by Butler and Jones (2) cites the report of Whitaker and Pryor (16) as the only reference to powdery mildew of lettuce.

Mr. L. L. Stubbs, in personal correspondence, said the disease has not been observed in Australia.

Pryor (10) reported powdery mildew infection of a wild-commercial-lettuce hybrid, No. 41858, but said that other such hybrids and commercial lettuce varieties growing nearby were not infected. This indicates that the strain reported by Pryor is not the same as that reported in 1951 by Snyder, Bardin, and Grogan (13), which was highly pathogenic to nearly all commercial head-lettuce varieties and strains. It seemed probable, however, that the powdery mildew found on cultivated lettuce since 1951 might be very similar in pathogenicity to the Salinas wild-lettuce mildew, possibly a mutant of it. Tests were therefore conducted to determine whether hybrids of wild lettuce and commercial lettuce were susceptible to the Salinas wild-lettuce mildew, and whether the Salinas wild-lettuce mildew differed from the powdery mildew now occurring on cultivated lettuce in the Salinas Valley.

Viable seed of hybrid 41858 was not available. Therefore, seeds of 16 hybrids related to hybrid No. 41858 (the lettuce breeding line on which powdery mildew was reported in 1940) were obtained from Dr. T. W. Whitaker. In gross appearance, these hybrids were similar to the common, crisp head-type lettuce. Pedigree data showed that all the hybrids were derived by crossing *L. serriola* from Europe with Imperial F and Imperial D, and later with Imperial 152. Most were in the 18–20th generation since the last cross.

A mildew isolate from cultivated lettuce in the Salinas Valley was maintained in stock culture on plants of Great Lakes lettuce grown in isolation in a

[1] Accepted for publication May 14, 1958.

[2] Respectively, Plant Pathologist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture; Associate Professor of Plant Pathology, University of California; and Plant Pathologist, Monterey County Department of Agriculture.

12°C refrigerator room. To ensure purity, single-conidial isolates of the wild-lettuce mildew from the Salinas Valley were established, since 2 strains of mildew occasionally occur together on wild-lettuce leaves in the Salinas Valley. Single spores on water agar were transferred by glass needle to detached wild-lettuce leaves (12). One of the single-spore isolates, selected and tested 6 times for pathogenicity on Great Lakes lettuce in detached-leaf culture, proved non-pathogenic. It is designated hereinafter as the Salinas wild-lettuce clone.

Tests were conducted in which detached leaves of 10 of the hybrids were inoculated with either the cultivated-lettuce strain or the wild-lettuce clone. Controls of these tests were uninoculated leaves of the hybrid lettuce strains that remained free of mildew, and wild lettuce inoculated with the wild-lettuce clone to determine whether this inoculum was viable and pathogenic to wild lettuce. The reactions of the hybrids to inoculation with the clone of the Salinas wild-lettuce strain were as follows: 46208-M and 45938-M were very susceptible; 45236-M and 46185-M were moderately susceptible; 45875-M was slightly susceptible; and 45325-M, 46162-M, 45814-M, 46189-M, and 46236-M were immune. These differences are similar to those reported by Whitaker and Pryor (16). All of the hybrids were very susceptible to the mildew from cultivated lettuce, and wild lettuce was susceptible to the Salinas wild-lettuce clone.

It appears, therefore, that the first authentic report of powdery mildew on lettuce (10) dealt with infection of wild-commercial hybrid 41858 by the Salinas wild-lettuce strain, and that the mildew on cultivated lettuce since 1951 is a distinctly different strain.

Distribution of cultivated-lettuce mildew.—Disease distribution was determined by surveys over a 2-year period—monthly in the Salinas Valley and irregularly in the fall-planted northern California lettuce areas. In addition, the Santa Maria district, south of the Salinas Valley (Fig. 2), was surveyed once, and a search for the disease was conducted in the Imperial Valley, in southern California.[3] Percentage of infected plants and severity of infection were determined by examining 20 randomly selected plants in a locality, recording the number of infected plants, and counting mildew colonies per basal leaf.

Up to February, 1953, lettuce powdery mildew had been reported only in the Salinas Valley (5). In the present investigation, however, the disease was found in the fall in lettuce-growing areas north of the Salinas Valley, near Aromas, Pajaro, Gilroy, San Jose, Concord, and Brentwood (Fig. 2). Powdery mildew was also found south of the Salinas Valley, near Parkfield, and near Yuma, Arizona, east of El Centro, California. The lettuce-growing area of the Salinas Valley, indicated on the map (Fig. 2), is mostly between Blanco and King City. Disease incidence and severity were greatest in the Salinas Valley, except that none was found in the coastal portion between Blanco and Monterey Bay (Fig. 2). Disease incidence and severity decreased with distance north of the Salinas Valley; none was found at Davis or Sacramento, the northernmost areas in the survey. The disease has probably been present in Arizona since 1953[4] (see Discussion).

FIG. 1. Severe case of lettuce powdery mildew from the field, showing A) leaf scorching, and B) basal leaf with profuse sporulation of the organism.

Host range of the cultivated-lettuce strain.—Mature potted plants or mature leaves in detached-leaf culture were used in the host-range studies.

All plants and leaves from seed were grown in isolation in a mildew-free greenhouse. The plants were dusted with sulfur and frequently checked for contaminating mildews. Plants and detached leaves to be inoculated were first washed free of sulfur. At least 3 potted plants and 3 detached leaves of each test host were inoculated. The tests were made in the

[3] The help of Dr. A. O. Paulus and of R. A. Kortsen and B. Hall is gratefully acknowledged.

[4] Personal correspondence with Drs. R. E. Foster and R. G. Marlatt, University of Arizona.

FIG. 2. Distribution, months of occurrence, and estimated percentage of infection of lettuce powdery mildew in California in 1954 and 1955. Disease rating was determined by evaluating the number of colonies per basal leaf. The disease also occurs in Arizona east of El Centro, California.

laboratory at about 22°C. except for a few tests in a greenhouse at 21–25°C. Continuous illumination of about 300 ft-c at the leaf surface was provided for plants and leaves inoculated in the laboratory. About 10 cc of a 3 per cent sucrose solution was used to float the detached leaves. Inoculations were made back to cultivated lettuce in triplicate from the hosts on which mildew growth was profuse enough to provide conidia.

Under laboratory conditions the host range of the fungus was found to be rather wide. Potted plants and detached leaves of the following species were susceptible: (Compositae) *Calendula officinalis* L. var. Double Mixed, *Dahlia variabilis* (Willd.) Desf. var. Unwin's Dwarf Hybrids, *Helianthus angustifolius* L., *H. annuus* L., *Lactuca serriola*, *Senecio cruentus* DC. var. Large Flowered Mixed, *Silybum marianum* Gaertn., and *Zinnia elegans* Jacq. vars. Floradale Scarlet and Giant Fantasy; and (Ranunculaceae) *Delphinium hybridum* Hort. var. Giant Imperial Blue Shades. Disease development was best on *D. variabilis*, *H. annuus*, *L. serriola*, *S. cruentus*, and *S. marianum*.

The following species became infected only in detached-leaf culture: (Compositae) *Cichorium intybus* L. var. Madgeburg, and *Cynara scolymus* L. var. Green Globe; (Cucurbitaceae) *Cucumis melo* var. *cantalupensis* Naud. var. S. R. 91, and *C. sativa* L. var. S. R. 6. In no case was a species susceptible as a potted plant but not susceptible in detached-leaf culture.

The following species were not susceptible, either as potted plants or detached leaves: (Begoniaceae) *Begonia tuberhybrida* Voss.; (Caryophyllaceae) *Dianthus barbatus* L. var. Single Mixed, and *D. chinensis* L. var. Double Mixed; (Compositae) *Baccharis halimifolia* L., *Callistephus chinensis* Nees var. Crego, *Centaurea cyanus* L., *C. solstitalis* L., *Cichorium endivia* L., *Grindelia squarrosa* (Pursh) Dunal, *Hypochoeris radicata* L., *Sonchus oleraceus* L., *and Taraxacum officinale* Weber; (Cruciferae) *Brassica hirta* Moench var. Large Smooth Leaf; (Cucurbitaceae) *Cucumis melo* L. var. Halis Best, *Cucurbita pepo* L. var. Early Sugar, and *C. pepo* L. var. *melopepo* Alef.; (Leguminosae) *Lathyrus odoratus* L.; (Linaceae) *Linum usitatissimum* L.; (Malvaceae) *Althaea rosea* Cav. var. Indian Spring; (Papaveraceae) *Eschscholzia californica* Cham.; (Polemoniaceae) *Phlox drummondii* Hook. var. Annual Scarlet; (Solanaceae) *Nicotiana tabacum* L. and *Solanum tuberosum* L. var. White Rose.

Deslandes reported that *Centaurea solstitalis*, *Hypochoeris radicata*, and *Taraxacum officinale* are susceptible to the lettuce powdery mildew strain (5). However, no mildew developed on these hosts in our tests. Species reported susceptible (5) but not tested in this study are *Baccharis douglassii* DC., *Grindelia camporum* Greene, and *Senecio vulgaris* L.

Tests to determine natural hosts of cultivated lettuce mildew were made as follows: Samples from various hosts were collected and each was placed in a plastic bag sealed against contamination with a rubber band. Conidia from each specimen were transferred on a sterilized dissecting needle to healthy detached lettuce leaves of the Great Lakes variety.

The powdery mildew that is pathogenic to Great Lakes lettuce was collected only from *L. serriola*, the only other natural host found in the field.

Powdery mildews not pathogenic to Great Lakes

TABLE 1.—*Comparative host ranges of several collections of E. cichoracearum from different host species in detached-leaf culture and on potted plants*

Mildew isolate from	Host and disease reaction[a]				
	Calendula officinalis	*Lactuca sativa*	*Lactuca serriola*	*Silybum marianum*	*Zinnia elegans*
Calendula	+	−	−	+	...
L. sativa	+	+[b]	+	+	+
L. serriola (Salinas)	−	−[b]	+	+	+
L. serriola (Davis)	...[c]	−	+	+	−
Zinnia	...	−	+	+	+

[a] Disease reaction was determined after 14 days' incubation at 22°C in the laboratory, with a minimum of 3 trials. "+" means the isolate was pathogenic; "−" means it was not.

[b] In some instances, collections of mildew from wild lettuce (*L. serriola*) have revealed the presence of mixed infections by the lettuce and wild-lettuce isolates.

[c] "..." indicates no measurements made.

TABLE 2.—*Comparative host range of Salinas wild-lettuce and cultivated-lettuce powdery mildew isolates in detached-leaf culture*

Host	Disease reaction to	
	Wild-lettuce strain	Lettuce strain
Calendula officinalis	0[a]	1
Cichorium intybus	1	1
Cucumis sativa	3	2
Dahlia variabilis	3	2
Delphinium hybridum	2	2
Helianthus angustifolius	2	2
Helianthus annuus	2	3
Lactuca sativa	0	3
Lactuca serriola	3	3
Senecio cruentus	3	3
Silybum marianum	3	3
Zinnia elegans	3	2

[a] Infection scale after 7 days' incubation at 22°C: 0, no visible signs; 1, infection but no sporulation; 2, infection with slight sporulation; 3, infection with profuse sporulation.

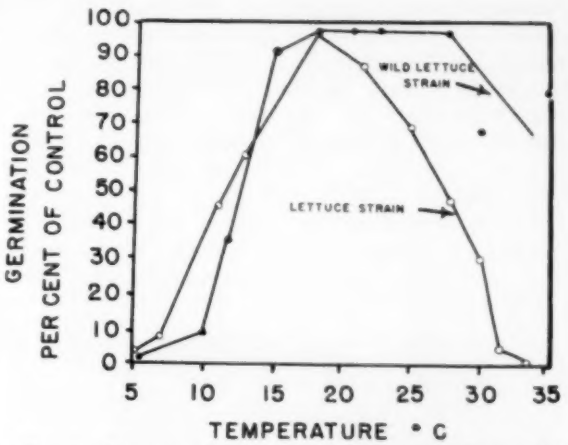

FIG. 3. Percentage of germination of the Salinas wild-lettuce and lettuce powdery mildew strains on glass slides at various temperatures. Each point represents at least 600 spores.

lettuce were collected from the following hosts: (Begoniaceae) *Begonia tuberhybrida;* (Compositae) *Calendula officinalis, Chrysanthemum* sp. L., *Coreopsis* sp. L., *Dahlia variabilis, Iva* sp. L., *Lactuca serriola, Matricaria* sp. L., *Senecio cruentus, Taraxacum officinale, Tragopogon porrifolius* L., and *Zinnia elegans;* (Hippocastanaceae) *Aesculus californica* Nutt.; (Hydrophyllaceae) *Phacelia* sp. Juss.; (Leguminosae) *Lathyrus odoratus;* (Papaveraceae) *Eschscholzia californica* and *Papaver* sp. L.; (Platanaceae) *Platanus* sp. L.; (Ranunculaceae) *Delphinium hybridum;* (Rosaceae) *Rosa* sp. L.; (Solanaceae) *Solanum* sp. L.; (Verbanaceae) *Verbena* sp. L.; and (Violaceae) *Viola tricolor* L. var. *hortensis* DC.

Over a period of several years, no powdery mildew was found on *Silybum marianum* in the field. *S. marianum* was susceptible only under conditions of modified environment, i.e. in the greenhouse or laboratory. This is in disagreement with Deslandes (5), who stated that he found abundant inoculum that infected lettuce on *S. marianum* in the field.

Pathogenicity tests by Deslandes (5) on lettuce with isolates of *Erysiphe cichoracearum* from other hosts were verified except that isolates of the wild-lettuce strain collected near Davis were not pathogenic to cultivated lettuce. Differences in host ranges of mildew collections from other hosts are shown in Table 1. Clear-cut differences were found among all isolates except the Salinas wild-lettuce mildew and the Zinnia mildew. However, the 2 strains were determined to differ physiologically by their reactions on certain wild-commercial-lettuce hybrids. No mildew developed on hybrids inoculated with the Zinnia isolate, whereas mildew was profuse when the Salinas wild-lettuce isolate was inoculated to the same hybrids.

Comparison of the cultivated-lettuce strain and the Salinas wild-lettuce clone.—1) *Comparative host ranges.*—Twelve species known to be susceptible to the lettuce strain of powdery mildew were inoculated with the 2 isolates used in inoculation tests of wild-

TABLE 3.—*Measurements of reproductive structures and their parts of the lettuce strain of E. cichoracearum with comparative conidial measurements of 2 isolates of the wild-lettuce strain*

Reproductive structures and parts	Mean (μ)		Range (μ)		Standard deviation (μ)		Number measured
	length	width	length	width	length	width	
Conidia							
Salinas wild-lettuce strain	32.7	15.8	24.8–38.3	9.9–19.8	±1.8[a]	±1.8[a]	150
Davis, wild-lettuce strain	32.5	15.9	22.9–42.0	12.6–18.2			50
Lettuce strain	37.0	18.1	28.1–58.5	11.5–27.2	±4.3[a]	±2.6[a]	150
Ascospores[b]	21.6	16.4	13.5–31.5	12.0–20.7			58
Asci	54.6	38.0	39.0–76.0	21.0–60.0			81
Stalks of asci	9.6	...[c]	3.0–19.5	...			69
Perithecia	99.1	99.1	...	89.1–148.5			100
Perithecial cells	18.0	12.3	9.0–40.7	7.5–16.5			18

[a] Significantly different at 1% level.
[b] Number of ascospores per ascus averaged 2, in range of 1–3 (Fig. 4).
[c] "..." indicates no measurements made.

commercial-lettuce hybrids.

The host ranges of the 2 isolates were strikingly similar, but they differed in pathogenicity on lettuce and in the severity of disease development on some other hosts (Table 2). The failure of the wild-lettuce clone to infect *Calendula officinalis* may not be significant, for infection of this host by the lettuce isolate also frequently failed.

2) *Comparison of the optimal temperatures for conidial germination of the 2 isolates.*—Fresh conidia of the 2 isolates (actually a clone of the wild-lettuce strain and several clones of the lettuce strain), dusted with a camel's-hair brush onto glass microscope slides mounted above 10 cc of distilled water in Petri dishes, were incubated in a series of temperature chambers regulated to vary no more than ±0.5°C.

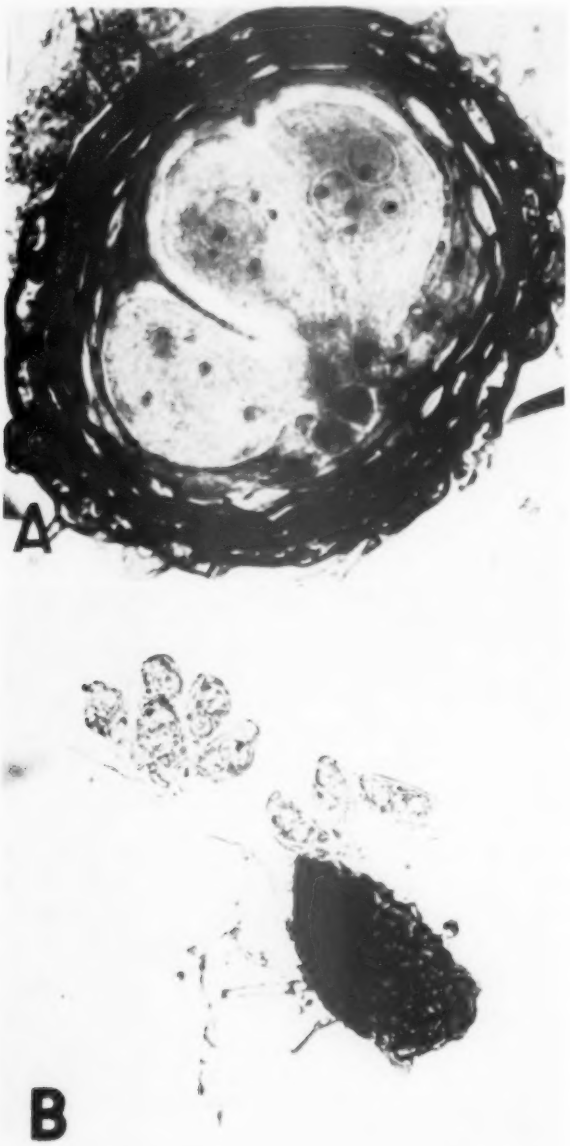

FIG. 4. Sexual stage of the lettuce powdery mildew strain. A) Longitudinal section through a perithecium showing arrangement of asci (note presence of 3 uninucleate ascospores per ascus). B) Broken perithecium and asci with 2 ascospores per ascus (the usual number). The appendages on the perithecium are mycelioid.

To facilitate comparison, germination percentages at the temperatures studied were plotted (Fig. 3) as per cent of the controls incubated at 18°C. Although the increase in germination with increase in temperature was about the same for both strains, the lettuce strain had a definite optimum at 18°C, whereas the wild-lettuce strain germinated equally well over a temperature range of 18–27°C. Germination of conidia of the lettuce strain decreased sharply above 18°C, and no germination occurred at 34°C. On the other hand, conidial germination of the wild-lettuce clone at 32°C was more than 70 per cent of that of the control. These data indicate that the lettuce isolate is more sensitive to high temperature than is the wild-lettuce isolate.

3) *Comparative morphological studies.*—Some features of the sexual stage of the lettuce isolate are shown in Fig. 4 as evidence that the cultivated-lettuce mildew fungus is *E. cichoracearum*. Measurements of the reproductive structures of the lettuce strain are given in Table 3.

Conidia of the wild-lettuce isolates obtained from the Salinas and Davis areas were not significantly different in size. Conidia of the cultivated lettuce isolate, however, were significantly larger than conidia of the Davis or Salinas collections of wild-lettuce mildew (Table 3). Whitaker and Pryor (16) reported a strain of wild-lettuce powdery mildew that had conidia considerably larger than those of either the Salinas or Davis wild-lettuce strains, and also larger than those of the cultivated-lettuce strain. There is evidently considerable variation in the size of conidia of strains that attack lettuce and wild lettuce, making conidial size a poor criterion for strain differentiation.

DISCUSSION AND CONCLUSIONS.—The distribution of powdery mildew of lettuce in California partly depends on the availability of susceptible hosts. However, spread of the disease to areas north of the Salinas Valley and its absence from the Blanco district of the Valley are probably largely due to the influence of environment. Several such environmental factors will be discussed in another paper. The isolated occurrence of lettuce powdery mildew near Yuma suggests that the disease was introduced there from the Salinas Valley; it is not known in the nearby Imperial Valley.

Powdery mildew of commercial lettuce appears to have originated independently in 2 widely separated parts of the world, Europe and the United States. It cannot be definitely stated that the European case was incited by the species that occurs in the United States, for no description of the sexual stage was given. A lack of valid reports of this disease elsewhere in the United States strongly indicates that the disease is presently restricted to California and Arizona.

It can be reasonably assumed that a disease will often occur each year in the area of geographic origin before it occurs in the rest of the region, that it will be most severe there, and that its life cycle will be consistently completed year after year. All of these events consistently occur in the Spreckels area of the Salinas Valley. If it is assumed that the lettuce strain is a mutant of the wild-lettuce strain, it is difficult to understand why the disease did not occur before 1951, since wild lettuce and its mildew are widely distributed in the United States (7, 15). It would seem that some recent agricultural practice in the Salinas Valley brought about the mutation, or that the mutation was spontaneous, and extremely rare. The 1940 occurrence of powdery mildew on hybrid 41858 may be of some significance in the evolution of the lettuce-mildew strain. It can be argued, however, that such a mutation could have occurred on wild lettuce or that the strain now attacking lettuce did not arise from the wild-lettuce strain, but from a strain of the fungus occurring naturally on some other host. It may be significant, or only a coincidence, that hybrid 41858 was planted in a variety trial not more than 1 mile from Spreckels, California.[5]

The data concerning the similarities in host ranges, together with the temperature sensitivity (3) of the lettuce strain, appear to favor the hypothesis that the lettuce strain is a mutant of the Salinas wild-lettuce strain. This hypothesis can be proved only by experimental production of such a mutant. The mutation probably involved a gain in ability to utilize an alternative host constituent, or a gain in ability to offset an inhibitory reaction exhibited by *L. sativa*.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
DAVIS, CALIFORNIA
AND
MONTEREY COUNTY DEPARTMENT OF AGRICULTURE
SALINAS, CALIFORNIA

[5] Personal correspondence with Drs. D. E. Pryor and T. W. Whitaker.

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INFLUENCE OF MOISTURE CONTENT, TEMPERATURE, AND TIME ON THE DETERIORATION OF STORED CORN BY FUNGI[1]

Subhi A. Qasem and Clyde M. Christensen[2]

SUMMARY

Moderate to heavy infection by storage fungi was common in 159 samples of corn stored 6–9 months in commercial bins; degree of invasion was roughly proportional to decrease in germination percentage and increase in percentage of discolored or damaged germs. High-quality yellow dent corn, inoculated with mixtures and individual species of common storage fungi isolated from corn, was stored at 5–25°C and 12–18% moisture content. All fungi tested were able to invade, kill, and discolor germs of corn stored above 10°C and 14% moisture content. Low temperature was as effective as low moisture content in preventing damage by the fungi tested. Germination percentage of control seeds kept free of fungi 8 months at 25°C and 18% moisture content decreased only slightly, and no brown germs developed; apparently, processes inherent in the seeds themselves were not involved in germ damage.

INTRODUCTION.—Fungi are a major cause of deterioration of wheat in commercial storage (3, 6, 7, 11), and the same is probably true of corn (2, 5, 8, 9). However, there is still relatively little information on the extent to which corn in commercial channels or in terminal bins is invaded by storage fungi, the influence of different storage fungi on decrease in germination and development of discolored or damaged germs, and the influence of moisture content, temperature, and time on the invasion of stored corn by the major fungi involved. Because corn is harvested and frequently stored at a higher moisture content than that of wheat, it is often subjected to a greater deterioration risk. This is a report on studies evaluating some of the factors involved in such deterioration.

MATERIALS AND METHODS.—Corn free of storage fungi was obtained from a planting of Minnesota hybrid No. 507 yellow dent and grown on test plots near St. Paul, Minnesota. Ears free of any obvious fungus infection or insect infestation were picked, dried, shelled, cleaned, and stored at 4–5°C and 40% relative humidity until used. After being shaken 1 minute in a solution of 1% sodium hypochlorite, rinsed in 2 changes of sterile water, and cultured on malt-salt and PDA (acid potato dextrose agars), 1% of the seeds yielded *Fusarium moniliforme* Sheldon, and less than 1% yielded Alternaria. No other fungi were seen. Germination of the seed averaged 99%.

Moisture content of the seed was determined by the one-stage or the two-stage air-oven method specified in *Cereal Laboratory Methods* (1), and all moisture contents are given on a wet-weight basis.

Germination percentage was determined by placing 100 seeds on moist paper towels, which were wrapped in waxed paper and incubated 9 days at room temperature, with counts made at intervals during this time. Seeds with well developed roots and coleoptiles were considered germinated.

For laboratory storage tests, 1000-g portions of seed were shaken 1–1.5 minutes in 1% sodium hypochlorite solution, rinsed twice in sterile distilled water, and dried aseptically in sterile paper bags in a forced-air oven at 30°C. After moisture content was determined, sterile water was added to the desired moisture content. The seeds at each moisture content were stored 5 days in sterile jars in a refrigerator and shaken occasionally to obtain uniform distribution of the moisture. Portions of 250–1000 g each were then placed in autoclaved pint or quart Mason jars equipped with screw caps and rubber rings, and stored in the appropriate incubators. A recording thermograph left 1 week in each incubator indicated that temperature fluctuated at times as much as ±2°C, but usually not more than ±1°C. Moisture contents, determined when the tests began and whenever portions were removed for sampling, varied no more than ±0.5%. Inoculum was furnished by pure cultures of the various storage fungi grown on autoclaved wheat; the cultures used had been isolated from commercial samples of corn.

Fungi present in the seeds were determined by shaking 50–100 seeds of each lot 1–1.5 minutes in 1% sodium hypochlorite plus 10 ppm of Tween-20, rinsing in 2 changes of sterile distilled water, and culturing on agar. Malt agar was used chiefly, but not exclusively. Other workers have found that this medium, compared with others, yielded more colonies of a greater number of fungi likely to be of significance in the deterioration of stored grains (2, 10). Tests preliminary to the present work were made with seeds from 5 different samples of corn and with 10 different agar media. The 5 corn samples showed varied de-

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[2] Research Assistant and Professor, respectively, Dept. of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, St. Paul, Minnesota.

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TABLE 1.—*Germination, germ discoloration, and fungus invasion of 159 samples of corn from commercial storage*

No. of samples	% germination		% germs discolored		% seeds yielding fungus[a]		
	Range	Av.	Ochre	Brown	Aspergillus flavus	Aspergillus glaucus	Penicillium species
25	0–30	17	18	28	27	25	38
35	31–60	49	18	8	2	36	23
99	61–95	80	6	3	2	27	9

[a] Seeds shaken 1 minute in 1% sodium hypochlorite, rinsed twice in sterile water, and cultured on malt-salt agar.

grees of invasion by common storage fungi. The media were malt agar containing 0, 50, 75, and 150 g of sodium chloride per liter; acid PDA containing 0, 50, and 75 g of sodium chloride per liter; and Czapek-Dox agar containing, respectively, 30 g and 200 g of sucrose per liter, and 200 g of sucrose and 50 g of sodium chloride per liter. One hundred seeds of each of the 5 lots were cultured on each of the 10 media, totaling 5000 seeds. More isolates of a greater number of storage fungi were yielded by the malt-salt agar containing 75 g of sodium chloride per liter, and by the Czapek-Dox agar containing 200 g of sucrose per liter, than by any of the other 8 media. The Czapek-Dox agar yielded a somewhat greater number of colonies of *Aspergillus flavus* Link than did the malt-salt agar, but the malt-salt agar yielded almost twice as many colonies of *A. repens* (Cda.) D By and other members of the *A. glaucus* Link group as did the Czapek-Dox agar, and nearly 10 times as many colonies of the *A. glaucus* group as did the acid PDA or the malt agar without salt. Since it seemed that the members of the *A. glaucus* group might be of considerable importance in the corn samples being investigated, it was reasonable to use a medium that would more readily reveal the presence of these fungi.

Mold count was determined by comminuting 10 g of seed (usually 40–50 seeds) in 500 ml of sterile 0.2% agar solution in water; 5 ml of this were placed in 45 ml of the same suspension medium and shaken 100 times. Further dilutions were made in the same manner, and 1-ml aliquots of 1 or more of these suspensions were put in each of 2 Petri dishes. Agar cooled to 50°C was added; the dishes were swirled, to distribute the material uniformly, and incubated 5–7 days; and the colonies were counted and identified.

Germ color of the seeds was determined by sectioning 100 seeds lengthwise through the middle of the germ and examining them with the unaided eye and through a stereoscopic microscope, using magnifications of ×10 and ×20. Germ color was rated as white (or pale yellow), ochre, brown, and blue. Grading regulations state that germs must be obviously discolored before being rated damaged; the ochre-colored germs probably would be rated sound by commercial inspectors.

RESULTS.—*Numbers and kinds of fungi in commercial samples of corn.*—One hundred and fifty-nine samples of corn that had been stored 6–9 months in commercial bins were tested. About half came from Minneapolis, Minnesota, and vicinity, 26 from Portland, Oregon, 16 from Omaha, Nebraska, 10 from St. Louis, Missouri, 6 from Chicago, Illinois, 6 from Granite City, Illinois, 40 from Marietta, Pennsylvania, 8 from Buffalo, New York, and 4 from Winston, North Carolina. Most were taken by probe 4–30 feet beneath the surface of the bulk, and comprised either about a pint or a quart of grain. Soon after received, they were tested for germination, germ discoloration, and storage fungi (Table 1).

The samples with the lowest percentage of seed germination had the highest percentage of seeds invaded by storage fungi, especially *Aspergillus flavus* and *Penicillium* species. It seemed likely that invasion of the germs of stored seeds by species of Aspergillus and Penicillium might be contributing largely to the development of discolored germs.

Inoculation of sound corn with storage fungi.—Yellow dent corn almost free of storage fungi (described in "Materials and Methods") was adjusted to moisture contents of 12, 14, 16, and 18%. Portions of 250 g each were placed in pint Mason jars and inoculated with a mixture of spores of *Aspergillus amstelodami* (Mano.) T. & C., *A. repens*, *A. ruber* (Spieck & Brem.) T. & C., *A. candidus*, *A. flavus*, *A. ochraceus* Wilh., and *Penicillium* sp. The jars were stored in incubators at 5, 10, 15, 20, and 25°C, and samples were periodically removed for testing.

No significant number of seeds stored at 12% moisture content yielded storage fungi at any time during 8 months. The percentage of seeds stored at 14% moisture content that were invaded by storage fungi (almost only *Aspergillus repens*) during 8 months increased fairly rapidly with increasing temperature, but germination percentage did not decrease appreciably, and no germs became discolored.

At 16% moisture content, increases in percentage of seeds invaded by fungi and percentage of discolored germs and decreases in germination percentage were slow at 5, 10, and 15°C, but rapid at 20 and 25°C (Fig. 1).

At 18% moisture content, invasion of the seed was rapid at all temperatures except 5°C, and there was a corresponding increase in percentage of discolored germs and decrease in germination percentage of the seeds (Fig. 2).

Uninoculated controls at 12, 14, and 16% moisture content retained a germination of 96% or more for 8 months, even at 25°C, and no germs became discolored. At 18% moisture content and 25°C, 92%

of the uninoculated controls still germinated after 8 months, although 16% of the seeds were invaded by Aspergillus (12% by *A. repens* and 4% by *A. flavus*). At the lower temperatures, the seed stored at 18% moisture content retained a germination of 96–100% for 8 months. At moisture contents up to 18% and temperatures up to 25°C, processes inherent in the seeds themselves apparently do not lead to appreciable deterioration. Also, a temperature of 5°C was just as effective as low moisture content in preserving high quality in stored corn as evaluated here. This is of some importance, since methods of cooling large bulks of grain are now available and in commercial use (4).

As stated above, seeds stored at a moisture content of 14% were invaded only by members of the *Aspergillus glaucus* group, chiefly by *A. repens*. Stored at 16% moisture content and 25°C, 34% of the seeds yielded *A. candidus* after 2 months, 42% after 3 months, and 60% after 4 months. This invasion by

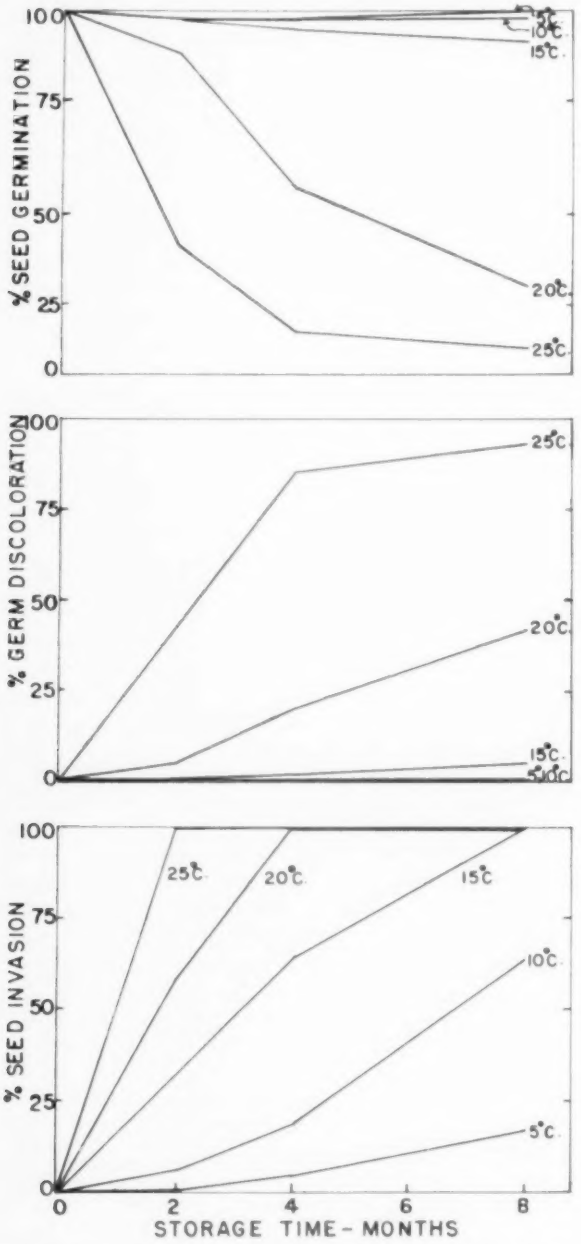

FIG. 1. Percentage of seeds invaded, germs discolored, and germination of yellow dent corn inoculated with a mixture of fungi and stored 8 months at 5–25°C and 16% moisture content.

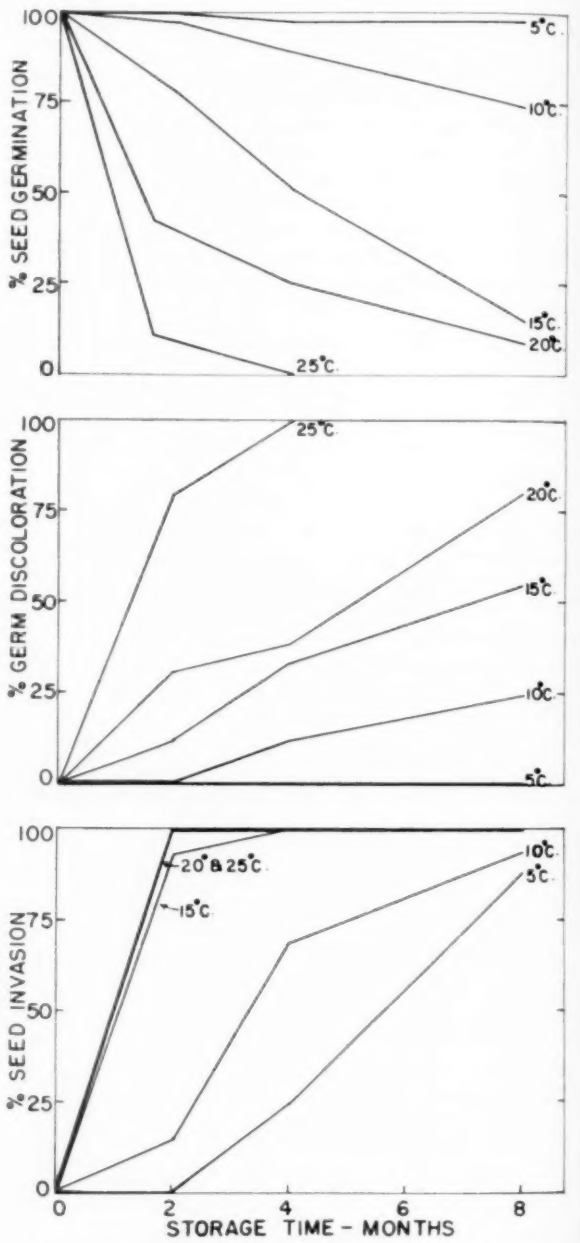

FIG. 2. Percentage of seeds invaded, germs discolored, and germination of yellow dent corn inoculated with a mixture of fungi and stored 8 months at 5–25°C and 18% moisture content.

TABLE 2.—*Germination, germ discoloration, fungus invasion, and perithecia formed in the interior of corn inoculated with 4 species of Aspergillus and stored at 18.5% moisture content and 20°C for 1, 2, and 3 months*

Inoculum	% germination	% germs discolored		% seeds invaded[a]	% seeds with perithecia
		Ochre	Brown		
At 1 month:					
A. amstelodami	90	6	2	84	8
A. repens	92	2	0	88	0
A. ruber	70	8	8	92	8
A. umbrosus	78	12	0	90	0
Uninoculated control	98	0	0	2[b]	0
At 2 months:					
A. amstelodami	68	12	0	92	12
A. repens	78	4	0	100	12
A. ruber	62	12	28	100	32
A. umbrosus	66	16	4	92	6
Uninoculated control	96	2	0	6[b]	0
At 3 months:					
A. amstelodami	45	48	18	100	32
A. repens	70	40	10	100	36
A. ruber	54	44	26	100	44
A. umbrosus	56	36	26	100	38
Uninoculated control	96	4	0	4[b]	1

[a] Seeds shaken 1 minute in 1% sodium hypochlorite, rinsed twice in sterile water, and cultured on malt-salt agar.
[b] Invaded by *A. repens*.

A. candidus was accompanied by a drastic decrease in germination percentage and a large increase in percentage of discolored germs.

Seeds stored at 18% moisture content were invaded chiefly by *A. repens*, *A. candidus*, and *A. flavus*. Decreasing germination percentage and increasing germ-damage percentage were associated mainly with invasion by *A. candidus* and *A. flavus*. Neither of these fungi invaded seeds stored at 5 or 10°C. Members of the *A. glaucus* group invaded 88% of seeds stored 8 months at 10°C and 18% moisture; 28% of the seeds were dead and 18% had ochre germs, but none had brown germs.

TABLE 3.—*Germination, germ discoloration, and fungus invasion of yellow dent corn inoculated with Aspergillus candidus and stored at 18% moisture content and 5, 15, and 25°C for 1, 3, and 6 months*

Months stored	Temperature °C	% germination	% germs discolored		% seeds invaded[a]
			Ochre	Brown	
1	5	100	0	0	0
	15	96	4	0	42
	25	76	12	16	100
3	5	98	0	0	0
	15	72	20	8	100
	25	2	24	32	100
6	5	98	0	0	0
	15	53	16	26	100
4.5	25	0	0	100	100
Uninoculated controls					
6	5	98	0	0	0
6	15	97	0	0	6[b]
4.5	25	94	2	0	8[b]

[a] Seeds shaken 1 minute in 1 per cent sodium hypochlorite, rinsed twice in sterile water, and cultured on malt-salt agar.
[b] *Aspergillus repens*.

A given degree of invasion of stored corn by different storage fungi apparently can cause widely different amounts of germ damage. *A. flavus* and *A. candidus* appear to be much more injurious than *A. repens* or other members of the *A. glaucus* group. All of these fungi invaded the germ preferentially, and often exclusively. A typical case is shown in Fig. 3.

To determine whether members of the *A. glaucus* group might, under conditions very favorable to their growth, cause germ damage in corn, yellow dent corn almost free of fungi was conditioned to a moisture content of 18.5% and different lots were inoculated with pure cultures of 4 members of the *A. glaucus* group most commonly isolated from commercially stored corn: *A. amstelodami*, *A. repens*, *A. ruber*, and *A. umbrosus* Bain & Sart. The samples were stored at 20°C and tested after 1, 2, and 3 months (Table 2). *A. ruber* caused somewhat more reduction in germination percentage, and more discolored germs, than did the other members tested. Uninoculated controls remained almost free of fungi; 4% of the germs became ochre and none became brown; germination after 3 months was 96%. Under conditions approach-

FIG. 3. Perithecia (arrows) of *Aspergillus ruber* formed in cavities in the germ and endosperm of corn seed stored 2 months at 20°C and 18% moisture content.

ing optimum for their growth, these members of the *A. glaucus* group evidently can cause considerable damage.

A. candidus was similarly inoculated onto essentially mold-free yellow dent corn at 18% moisture content, and 250-g portions were stored at 5, 15, and 25°C and tested after 1, 3, and 6 months (Table 3).

At 6 months, *A. candidus* had not invaded any of the seed stored at 5°C. At 1 month it had invaded 42% of the seeds stored at 15°C, and 100% of those at 25°C. At 3 months it had invaded all the seeds stored at 15°C. A decrease in germination percentage and increase in percentage of discolored germs accompanied or closely followed invasion of the germs by this fungus. A few of the uninoculated control seeds were invaded by *A. repens*, but germination percentage decreased only very slightly, only 2% of the germs became ochre, and none became brown. If conditions are at all favorable for its growth, *A. candidus* evidently can rapidly invade, kill, and discolor the germs of even such high-grade corn as was used here. It is known to cause similar damage in stored wheat (6).

Men in the grain trade have claimed that different lots of No. 2 yellow corn differ greatly in storage behavior. Under conditions favoring deterioration, some lots develop germ damage much more rapidly than do others. In our examination of corn lots from commercial storage, some samples of No. 2 yellow corn had a much higher percentage of ochre germs than did others. These ochre germs usually were heavily invaded by various storage fungi. It seemed that this might offer a clue to different storage behavior of different lots under essentially the same conditions. To test this, 3 lots of No. 2 yellow corn were selected. They differed very little in percentage of damaged (brown) germs, but differed considerably in percentage of ochre germs, as well as in percentage of germination and in mold count. All were of the 1956 crop, stored 1 year in different commercial bins in the Midwest. For comparison, a sample of the almost-mold-free corn used in the laboratory tests was included; this also was 1 year old. The 4 samples were conditioned to a moisture content of 18%, stored 30 days at 25°C, and then tested for germination, germ color, and mold count (Table 4).

The lot of commercial corn that originally had the lowest germination percentage, the largest number of ochre germs, and the largest mold count, deteriorated much more, as measured by these criteria, than did the other 2 lots of commercial corn; and these others deteriorated much more than did the originally almost-mold-free lot of high-grade corn, which, stored 1 month under these severe storage conditions, retained a germination of over 90%. Many factors may influence the rate at which corn deteriorates under a given set of conditions in storage, but an important factor is certainly the degree to which the germs have already been invaded by various fungi.

Discussion.—Storage fungi do not invade the seeds of wheat or barley before harvest to any great extent (10, 11). Our evidence with corn on this point is not extensive enough to warrant tabular presentation, but has been consistent. Seeds of 20 different collections of corn standing in the field in Minnesota during the moist post-harvest season in 1957 were cultured, after being shaken 1 minute in 1% sodium hypochlorite, and very few of them yielded storage fungi although many colonies of Aspergillus and Penicillium grew from the surface of seeds that were cultured without this prior treatment. The same has been true of many samples of presumably freshly harvested seed obtained from commercial handlers. Evidently, corn at harvest is relatively free of deep infection by common storage fungi.

Table 4.—*Germination, germ discoloration, and mold count of 4 samples of corn before and after storage for 30 days in the laboratory at 18% moisture content and 25°C*

	% germination	% germs discolored		Mold Count (thousands)
		Ochre	Brown	
Before storage:				
Commercial bin #1	50	16	1	7.5
Commercial bin #2	62	11	2	11.5
Commercial bin #3	48	22	5	81
High-grade seed	98	0	0	4
After storage:				
Commercial bin #1	2	38	13	1830
Commercial bin #2	0	42	12	1180
Commercial bin #3	0	43	25	10,600
High-grade seed	94	4	0	750

Moderate to rather heavy infection was common in samples that had been 6–9 months in commercial storage, indicating that moisture and temperature conducive to such invasion must have prevailed at some time during their storage life. Invasion of the seeds from commercial bins by Aspergillus and Penicillium, particularly by *A. flavus*, was roughly proportionate to decrease in germination percentage of the seed and to increase in percentage of discolored germs.

All of the fungi tested were able to invade, kill, and discolor the germs of corn seed stored 8 months at moisture contents above 14% and temperatures above 10°C. At a given degree of invasion, *A. candidus* and *A. flavus* were more injurious than the *A. glaucus* group. In that group, *A. ruber* caused more decrease in germination percentage and a greater increase in percentage of discolored germs than did *A. amstelodami*, *A. repens*, or *A. umbrosus*, though the differences were not great. Different isolates of *A. candidus* differed widely in the amount of sick or germ-damaged wheat they caused under uniform storage conditions (6), and it is quite possible that, if enough isolates were tested of the different subspecies of the *A. glaucus* group, similar differences might be found within each of them. The lower limit of moisture content that permitted invasion was 12–14% for the *A. glaucus* group, 14–16% for *A. candidus*, and

16–18% for *A. flavus*. Thus, if a considerable percentage of seeds in a given lot of corn from commercial storage yield *A. flavus* after surface disinfection, it is reasonable to suppose that the seed has been stored at a moisture content approaching 18%; and if many of the seeds yield *A. candidus* it may reasonably be supposed that the seeds have been stored for a time at a moisture content close to 16%. That is, the mold flora in a given lot of seed furnish at least a partial record of past storage conditions, as well as an indication of future storage risk. It seems probable that some of the methods developed to evaluate the moldiness of stored corn might be used to augment present inspection procedures in judging the quality and storability of corn.

In all of the tests here reported, invasion of the corn germs by fungi preceded death of the germs, and death of the germs preceded development of dark color in the germ. This is the same course of events found in the development of sick or germ-damaged wheat in commercial storage and in the laboratory (3, 6, 7). There seems little doubt that, in practice, fungi are a major cause or contributing factor in the development of germ damage in commercial corn.

Numerous control samples were maintained free or almost free of storage fungi for as long as 8 months, and even when these had 18% moisture content and were stored at 25°C there was little decrease in germination percentage of the seed, and no brown germs developed. This indicates that, under the conditions of the test, processes inherent in the seeds themselves were not significantly involved in the production of germ damage.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY
INSTITUTE OF AGRICULTURE
UNIVERSITY OF MINNESOTA
ST. PAUL 1, MINNESOTA

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THE EFFECT OF PRE-INOCULATION TEMPERATURE ON TEST PLANT SUSCEPTIBILITY TO ALFALFA AND TOBACCO MOSAIC VIRUSES[1]

J. D. Panzer

SUMMARY

Phaseolus vulgaris plants proved more resistant to alfalfa mosaic virus when conditioned for 2 hours immediately before inoculation at low or high temperatures than at moderate temperatures. They were also more resistant to tobacco mosaic virus when conditioned at low than at moderate or high temperatures. *Nicotiana tabacum* and *N. glutinosa* were susceptible to tobacco mosaic virus at all of these temperatures held for 2 or 6 hours. Plant resistance imposed by the temperatures was of short duration. Such temperatures are held to affect the condition of the plant and not the virus. Thermal inactivation of alfalfa mosaic virus in solution required 5 days at 5°C and 15 minutes at 35°C.

Temperatures have been demonstrated to influence virus disease expression in plants. Low or high temperatures following inoculation have increased disease development (2, 3, 7, 10), decreased disease development (3, 7, 10), modified symptoms (4, 8, 10), or eliminated the virus (6). High temperatures before inoculation have increased (5, 9) or decreased (1) disease development. Low temperatures before inoculation appear not to have been investigated.

Since plants undergo large changes in leaf temperature during the course of a day it is possible that plants would be differentially susceptible to inoculation-infection directly, as related to high and low leaf temperatures before inoculation. To provide a partial answer to this possibility, greenhouse experiments with alfalfa and tobacco mosaic viruses were conducted with bean and tobacco assay plants. All but 1 assay plant produced local lesions. All were conditioned 2 hours at different temperatures before being inoculated.

MATERIALS AND METHODS.—The test plants were 4 *Phaseolus vulgaris* varieties—Pinto, Bountiful, Yellow Eye, and Red Kidney—and plants of *Nicotiana glutinosa* and *N. tabacum*. AMV (alfalfa mosaic virus) was obtained from infected alfalfa plants collected in South Dakota; TMV (tobacco mosaic virus) was furnished by Dr. L. J. Alexander, of the Ohio Experiment Station. The AMV consisted of 2 strains; one produced large local lesions on bean plants; the other produced small lesions.

Bean plants (12–14 days old) and tobacco plants (7–10 weeks old) growing in steamed soil at 20°C under greenhouse conditions were left 2 hours in the dark in controlled-temperature cabinets at temperatures ranging from 5 to 50°C at 5° increments. Immediately after, 2 primary leaves of beans or 3 of the lower leaves of *N. glutinosa* were dusted with #600 grit Carborundum and swabbed with a virus inoculum consisting of juice expressed from diseased alfalfa or tobacco plants and mixed with a 10% solution of K_2HPO_4. The plants were then returned to the greenhouse, and infection was allowed to develop. The numbers of lesions per leaf were recorded. In all cases the data were subjected to a variance analysis.

RESULTS.—Some plants were injured at 50°C, but none appeared injured at the other temperatures.

Beans as assay plants.—Four experiments, each replicated at least 3 times (24 leaves per treatment), were conducted with AMV strain 1, AMV strain 2, and TMV.

1) AMV strain 1.—The average numbers of lesions per leaf in 4 experiments on each of 3 bean varieties, Yellow Eye, Bountiful, and Red Kidney, are presented in Fig. 1A.

Lesion numbers increased with temperature up to about 20°C, and then decreased. The behavior shown in Fig. 1A was evident in each of the 4 experiments. The greatest number of lesions developed at 15–30°C. Numbers decreased markedly below 15°C.

2) AMV strain 2.—The average numbers of lesions per leaf in 4 experiments with Yellow Eye bean inoculated with AMV strain 2 are presented in Fig. 1B.

Similarly as with strain 1, lesion numbers increased with temperature up to 35°C, and then decreased. The greatest number of lesions developed at 15–40°C. Numbers decreased markedly below 15°C.

3) TMV.—The average number of lesions per leaf in 4 experiments with Pinto bean plants inoculated with TMV are presented in Fig. 1C.

As with bean plants inoculated with AMV, lesion numbers generally increased with temperature up to 25°C, beyond which the reactions varied.

Nicotiana species as assay plants.—Paralleling the above experiments on beans were experiments with TMV on 2 tobacco species.

1) *N. glutinosa.*—The average numbers of lesions developing per leaf from TMV in 4 experiments, each replicated 3 times (12 leaves per treatment), are presented in Fig. 1C. Temperature did not significantly affect the number of lesions.

2) *N. tabacum.*—Plants of this species were tested for successful or unsuccessful inoculation in 2 experiments of 2 replications each, similarly to the above.

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All plants were infected by inoculation, regardless of temperature treatments, and all results were similar.

Because a 2-hour treatment did not result in resistance, and because Allington, Weihing, and Laird (1) found that excised leaves of *N. tabacum* became resistant to infection by TMV only after a 10-minute pre-inoculation exposure to 45°C followed by a 6-hour incubation period at the same temperature, the treatment period was increased to 6 hours.

Five *N. tabacum* plants were exposed to 45°C for 6 hours. Another set of plants was exposed to 20°C for the same period. After the heat treatment the plants were immediately inoculated with TMV and returned to the greenhouse, where infection was allowed to develop. All the plants became infected. Thus, entire plants exposed only to high pre-inoculation temperatures did not react as did the excised leaves of Allington, Weihing, and Laird (1), which had received both pre- and post-inoculation heat treatments.

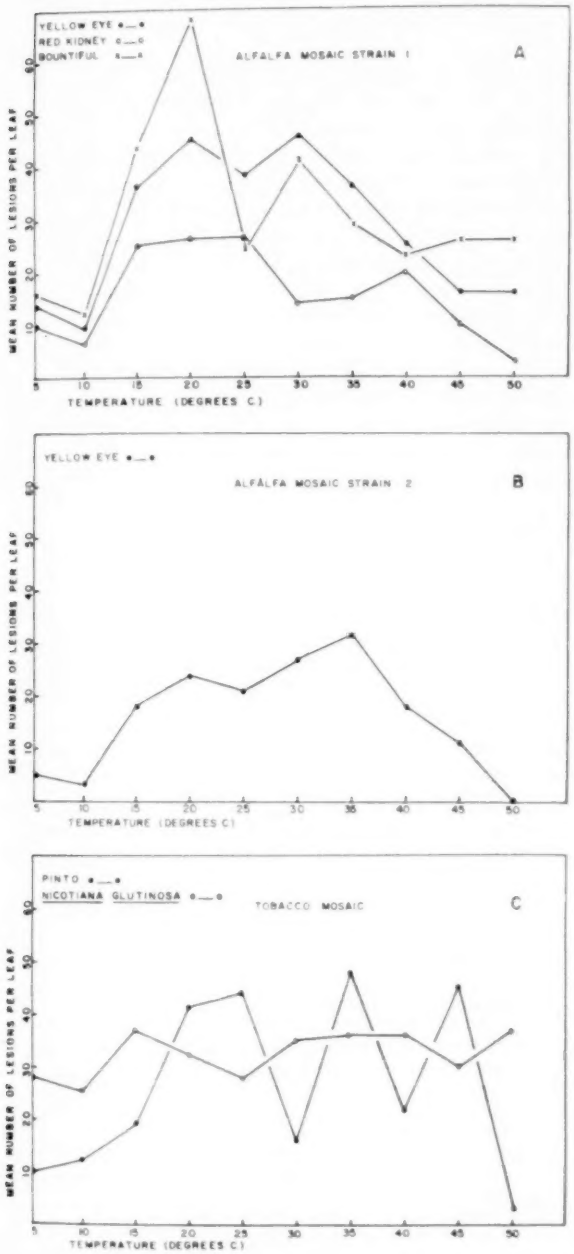

FIG. 1. Mean number of lesions per leaf of test plants receiving different temperature treatments before inoculation with A) alfalfa mosaic virus strain 1; B) alfalfa mosaic virus strain 2; or C) tobacco mosaic virus.

Duration of the pre-inoculation temperature effect.—The duration of the effect of pre-inoculation temperature treatments was determined by subjecting bean plants to pre-inoculation temperatures of 10° and 20°C, and inoculating immediately, 15 minutes, 30 minutes, or 1, 2, 3, 4, 5, or 6 hours after treatment. The usual reduction in the number of lesions (3-fold) found at the 10°C treatment occurred only if the plants were inoculated immediately. As little delay as 15 minutes nullified the treatment, resulting in no significant reduction in number of lesions.

Measurements of leaf temperature were taken by means of a copper-constantan thermocouple taped to the undersurface of the bean leaves. It was found that temperature dropped about 6°C in the first 60 seconds after plants were removed from the heat treatments and placed under room conditions. If plants were inoculated as soon as 15 seconds after removal from the heat chambers, their leaves reached room temperature 1 minute later.

Direct temperature effects on the inoculum.—The above results of reduced infectivity of virus in plants predisposed at low or at high temperatures may have arisen from a modification of either the host or the inoculum, or both. A modification of the inoculum seems possible since the plants were inoculated immediately upon removal from the temperature cabinets. However, since TMV is known to be stable to these and higher temperatures and because AMV is known to be relatively unstable, the immediate concern would appear to be whether AMV had been directly inactivated by the temperature treatments.

To test this possibility, vials containing 10 ml of an AMV-phosphate solution were placed in the dark in controlled-temperature cabinets for periods ranging from 15 minutes to 5 days and temperatures ranging from 5° to 55°C. The virus was then assayed for infectivity by swabbing it onto the surface of the primary leaves of Bountiful bean. The results are presented in Table 1.

Temperature and its duration had a direct effect on AMV infectivity. Temperatures of 35°C or greater inactivated the virus within 15 minutes. The data are inadequate to show the inactivation time of the virus at temperatures above 35°C for periods shorter than 15 minutes. Consequently, no statement can be made on reduction in susceptibility in bean plants exposed to high-temperature treatments directly before inoculation. At low temperatures, however, the treatments would appear to affect the host rather

TABLE 1.—*Infectivity*[a] *of a strain of alfalfa mosaic virus after storage in phosphate solution at various temperatures and periods*

	Exposure Time											
	Minutes							Days				
°C	15	30	45	60	120	180	480	1	2	3	4	5
5	+	+	+	+	+	+	+	+	+	+	±	—
10	+	+	+	+	+	+	+	+	±	—	—	—
15	+	+	+	+	+	+	+	—	—	—	—	—
20	+	+	+	+	—	—	—	—	—	—	—	—
25	+	+	+	+	—	—	—	—	—	—	—	—
30	±	±	—	—	—	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—	—	—	—	—	—
40	—	—	—	—	—	—	—	—	—	—	—	—
45	—	—	—	—	—	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—	—	—	—	—	—
55	—	—	—	—	—	—	—	—	—	—	—	—

[a]Infection scale: + good infection; ± slight infection; — no infection.

than the virus directly. The reduction noted at lower-temperature pre-inoculation treatments cannot be explained by a direct temperature effect on the virus, since the exposure of inoculum to the temperatures existing in the leaves of the treated plants at the time of inoculation was too brief to inactivate the virus.

Since TMV is relatively stable at temperatures up to 85°C and as low as —23°C, the reduction in lesion number on Pinto beans receiving low-temperature treatments may be attributed to an effect on the host.

DISCUSSION.—Pre-inoculation temperatures appear to play an important part in the susceptibility of bean plants to certain virus pathogens. Susceptibility seems related not only to virus specificity but to plant temperature at inoculation time. In these tests the most sriking effect occurred at the lower temperatures, where a sharp development of resistance was noted at 10°C. This low-temperature resistance occurred whether the virus was stable (TMV) or nonstable (AMV) under thermal-inactivation treatment. It is explained as an effect of temperature on the host rather than on the virus.

At higher temperature treatments, however, thermal inactivation of the virus itself could be a factor.

In these experiments entire plants received the heat treatments, and the duration was 2 hours. The results obtained with TMV on *N. tabacum* were in contrast to those reported by Allington, Weihing, and Laird (1), for infection occurred at all temperatures with pre-inoculation exposures of 2 or 6 hours. Since those workers exposed leaves to both pre- and post-inoculation treatments, it appears that the heat-induced resistance they noted was due to the post-inoculation and not the pre-inoculation temperature treatments. The studies with TMV at the high temperatures were not conclusive for temperature effects on bean although the work with AMV at these temperatures indicated a decrease rather than an increase in susceptibility, as noted by Yarwood (9) with many viruses and fungi (AMV was not included). Whether this was due to the specific nature of the AMV or to the fact that the entire plants were exposed for a long period, cannot be stated at this time.

It is suggested that pre-inoculation low or high temperatures affect the plant so as to render it less suitable for establishment of the virus.

Allington, Weihing, and Laird (1) suggested that decreased susceptibility at the higher temperatures might support the hypothesis that TMV infection proceeds along lines similar to those now recognized for bacteriophages, with the nucleic acid thermolabile at the time of infection. The low temperature resistance found by the author could not support the thermolabile theory, although the mechanism for resistance may differ at high and low temperatures, and in a local-lesion host like bean is probably different from the mechanism in a systemic-infection host such as *N. tabacum*. If infection by plant virus diseases proceeds along lines similar to that by bacteriophages, the infection process in bean would seem to depend on some process of the host that would occur at a relatively critical temperature, above and below which the process would not take place.

DEPARTMENT OF PLANT PATHOLOGY
SOUTH DAKOTA STATE COLLEGE
BROOKINGS, SOUTH DAKOTA

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THE CONTROL OF SOAKING INJURY OF BARLEY SEED[1]

S. H. F. Chinn and R. C. Russell[2]

SUMMARY

Tests of barley seed treated by the water-soak method to control loose smut show that the treatment impaired germination. However, soaking injury was substantially reduced by adding sodium chloride to the water, 1 or 2% being slightly more beneficial than 3%. Ammonium phosphate and sodium citrate were as effective as sodium chloride; mercuric chloride and silver nitrate were more effective; ascorbic acid, sodium sulphate, and others were less effective; certain others, especially ammonium nitrate, glucose, and sucrose, increased soaking injury.

In general the germinative capacity of seeds is impaired by prolonged soaking in water at room temperature. Such "soaking injury" has been reported, in studies with beans and other seeds, by Kidd and West (7), Tilford, Able, and Hibbard (10), Barton (3), Bailey (2), Barton and MacNab (4), and others. Working with the water-soak method specifically for the control of loose smut of barley, Tyner (11), Russell and Tyner (9), Arny and Leben (1), and Machacek and Wallace (8) also observed the soaking injury. Russell and Tyner (9) found that the amount of injury increased regularly with soaking time and temperature. They reported that, though soaking is effective for eradicating loose smut of barley, the treatment is little used by barley growers, because of the reduced germination and extra labor.

In attempts to develop methods that are both effective and practical for control of loose smut, the writers found that adding sodium chloride to the water-soak reduced soaking injury significantly. This prompted a series of investigations on the effect of sodium chloride and other chemicals on soaking injury; the results are reported herein.

The writers have since learned that Ivanoff (6) has reported that soaking oat seeds in salt water produced healthier seedlings than soaking in plain water. He suggested that salts at certain osmotic concentrations eliminated "weak" seeds, causing death through cell plasmolysis.

MATERIALS AND METHODS.—Barley seed was soaked at room or controlled temperatures for specified periods in water or water containing various chemicals. The soaked seed was dried, and sown in the greenhouse at the rate of 300 seeds per flat, and in the field at 125 seeds per 8-foot row replicated 4 times. Germination was rated on emergence, and recordings were made when the seedlings were in the two-leaf stage. In both greenhouse and field tests dry untreated seed was sown for comparison. Emergence in the untreated checks was considered 100% for computing germination and injury percentages.

EXPERIMENTAL RESULTS.—*Water-soak injury to barley seed.*—Four samples of Rex barley, 7 of Husky, 6 of Montcalm, and 3 of Warrior were tested for susceptibility to soaking injury. The seeds were soaked 65 hours at 24°C and sown in the greenhouse. All seed lots showed injury (Table 1), ranging from 13%, for a sample of Montcalm from Balcarres, to 98%, for a sample of Rex seed from Melfort. Since only a few samples of 4 varieties were tested, it is not possible to draw conclusions as to varietal differences in susceptibility to soaking injury. The data show, however, the existence of intravarietal differences, as witness the 13–97% range of injuries in Montcalm samples.

Effect of sodium chloride on soaking injury.—1) *Greenhouse trials.*—Numerous samples of different varieties of barley were tested for susceptibility to injury from soaking 65 hours in water or 1 or 2% sodium chloride solution at 24°C. Four typical samples were injured in varying degrees by the water-soak (Fig. 1). Sodium chloride significantly reduced injury. The 1% solution reduced the respective injury percentages from 83 to 23, 66 to 32, 49 to 25, and 12 to 0. The 2% solution showed a greater reduction of injury in 2 samples, and a lesser in the other 2.

2) *Field trials.*—One seed sample from each of 4 varieties of barley was tested for susceptibility to injury by soaking 55, 65, or 75 hours in water or 1, 2, or 3% sodium chloride solution at room temperature. The seeds were tested for germination in field plots at Saskatoon and Scott. Averages of results from the 2 locations (Fig. 2) show that all 4 samples were injured regardless of the length of soak, varying in general with length of soak. Some samples were more severely injured than others. The Gateway received minimal injury (7%) even at the 75-hour period. At the other extreme the Rex seed was injured severely (64%) at the 55-hour period. Sodium chloride was particularly beneficial in treatments that caused heavy injury. With some exceptions, 1% addendum was slightly more effective than 2%; 3% was least effective. Sodium chloride was not significantly beneficial in soak treatments that caused slight injury. In some cases, especially at the 3% level,

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[2] Respectively, Bacteriologist and Plant Pathologist.

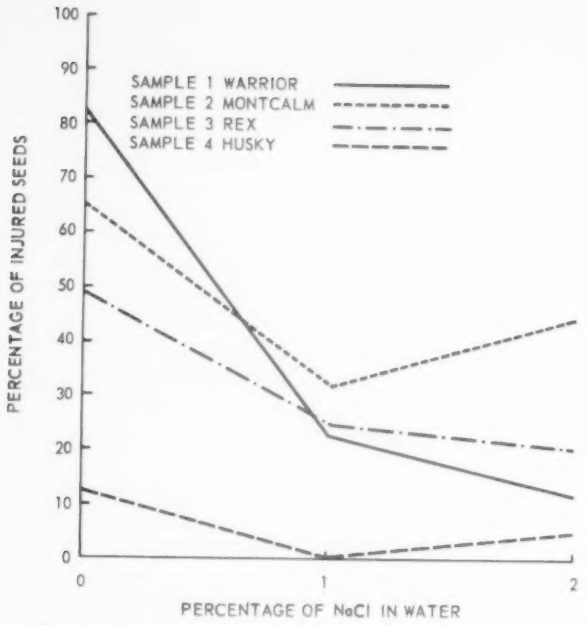

FIG. 1. Injury, as shown by germination under greenhouse conditions, of 4 varieties of barley seeds soaked 65 hours at 24°C in water or in 1 or 2% sodium chloride solution.

injury was slightly increased.

Effect of various chemicals on soaking injury.—An experiment was conducted to determine whether chemicals other than sodium chloride were capable of reducing soaking injury. The seed used in the study was a sample of Montcalm that tested about 75% germination and was very susceptible to soaking injury. It was divided into a number of lots, which were soaked 72 hours at 24°C in water or water containing the various chemicals. Germination tests were made in the greenhouse. Seeds soaked in water were injured to the extent of 89% (Table 2). Of the 17 chemicals tested, 12 reduced and 5 increased soaking injury. Mercuric chloride and silver nitrate were the most effective, the former eliminating soaking injury completely. Sodium citrate and ammonium phosphate were as effective as sodium chloride; 7 chemicals were less effective; 5 intensified soaking injury—sodium acetate and l-cystine but slightly, and ammonium nitrate, glucose, and sucrose to complete suppression of germination.

DISCUSSION.—It is evident that the germinative capacity of barley is impaired, often very severely, by prolonged soaking in water at room temperature. Whether varietal differences exist in susceptibility to soaking injury cannot be concluded from these restricted studies. However, wide differences in response occurred in samples within varieties. This, along with the fact that some of the older seed samples were injured less than newer samples, suggests that condition of the seed is important.

The results presented here show that the ill effects of the water-soak can largely, if not entirely, be eliminated by adding certain chemicals to the water. Choice is yet to be made among mercuric chloride, which gave complete reduction in soaking injury; silver nitrate, which gave high reduction; and sodium chloride, which gave moderately high reduction. Experiments not yet reported show that control of loose smut is not lessened by sodium chloride at concentrations of 1 and 2%, mercuric chloride at 0.02%, or

TABLE 1.—*Effect on 20 samples of barley seed of water-soaking 65 hours at 24°C*

Variety	Where and when obtained	Actual % germination: Not soaked	Actual % germination: Soaked	% injury[a]
Rex	Unknown, 1949	87	51	41
	Melfort, 1952	45	1	98
	Saskatoon, 1953	90	9	90
	Melfort, 1953	84	31	63
Husky	Saskatoon, 1953	87	42	52
	Tisdale, 1954	99	74	25
	Kelvington, 1953	99	81	18
	Saskatoon, 1955	81	34	58
	Nipawin, 1956	85	47	45
	Larbe, 1956	85	62	27
	Lorlie, 1956	79	22	72
Montcalm	McNutt, 1949	77	2	97
	Melfort, 1950	55	2	96
	Nut Mountain, 1954	73	35	52
	Balcarres, 1956	93	81	13
	Unknown	71	24	66
	Gunton, 1956	76	8	89
Warrior	Kelvington, 1951	83	14	83
	Indian Head, 1953	96	54	44
	Unknown	80	14	83

[a] Emergence in the untreated check was considered as 100% germination.

TABLE 2.—*How various chemicals added to the water influenced the injury of Montcalm barley soaked 75 hours at 24°C*

Chemical	% chemical in water	% injury[b]
Untreated check		0
Water only		89
Mercuric chloride	0.02	−1
Silver nitrate	0.1	3
Sodium citrate	1	20
Ammonium phosphate	1	23
Sodium chloride[a]	1	25
Lithium chloride	1	34
Ammonium chloride	1	34
Calcium chloride	1	34
Ascorbic acid	1	46
Sodium sulphate	1	47
Ferric chloride	1	56
Potassium chloride	1	67
Sodium acetate	1	93
l-cystine	1	94
Ammonium nitrate	1	100
Glucose	1	100
Sucrose	1	100

[a] Commercial non-iodized salt; all other chemicals in the experiment were chemically pure.

[b] Emergence in the untreated check was considered as 100% germination.

silver nitrate at 0.1%. The data on soaking injury, alone or combined with those on the control of loose smut, indicate the superiority of mercuric chloride and silver nitrate over sodium chloride. However, sodium chloride, the commercial non-iodized type, is economical and readily available; it is not as dangerous as the other 2 chemicals for use on the farm. An objection to the water-soak treatment is the foul odor during soaking and drying. Any of the above 3 chemicals made the odor less objectionable.

It is interesting to speculate on the cause of soaking injury and the mechanism of reduction of soaking injury by sodium chloride. Barton and MacNab (4), in a study of the injury of bean seed soaked in water supplied with oxygen, found that injured seeds absorbed excessive amounts of water. Eyster (5) concluded that losses of proteins, digestive enzymes, and growth-promoting substances were the main factors responsible for the decreased germination of bean seeds soaked in water. However, he did not entirely

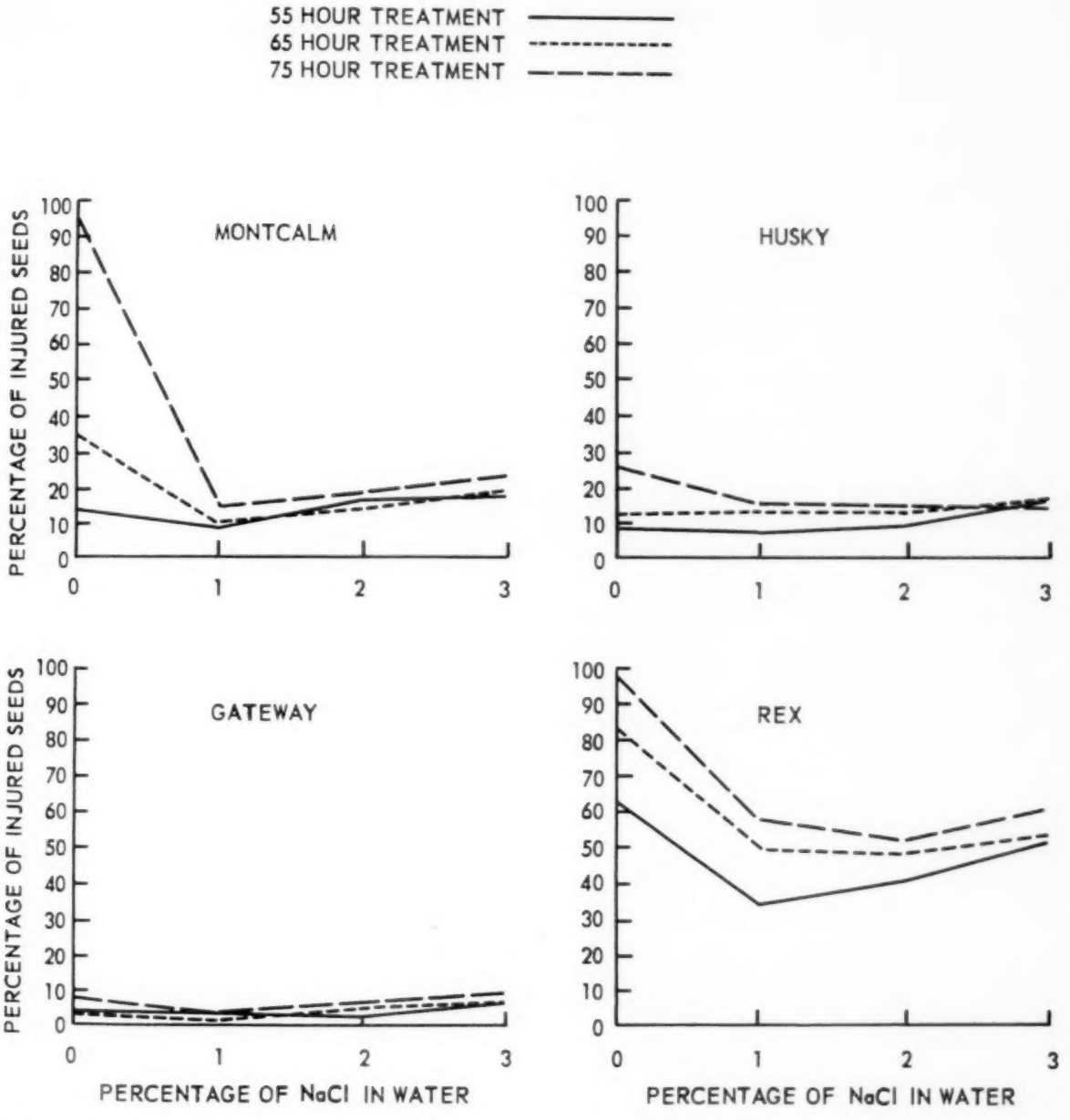

FIG. 2. Injury, as shown by germination under field conditions, of 4 varieties of barley seeds soaked 55, 65, or 75 hours at room temperature in water or in 1, 2, or 3% sodium chloride solution.

ignore the role of bacterial activity in the devitalization of bean seeds. The factors responsible for soaking injury in barley are being investigated.

PLANT PATHOLOGY SECTION
CANADA DEPARTMENT OF AGRICULTURE RESEARCH LABORATORY
UNIVERSITY SUB POST OFFICE
SASKATOON, SASKATCHEWAN, CANADA

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HISTOLOGY OF CERTAIN OAKS INFECTED WITH THE OAK WILT FUNGUS[1]

B. Esther Struckmeyer, J. E. Kuntz, and A. J. Riker

SUMMARY

Fungus mycelium was observed rarely in the xylem vessels of infected northern pin oaks before or during incipient wilt. During severe foliar wilt, mycelium developed extensively inside the vessels. The fungus grew outward by penetrating the bordered pits to adjacent ray and xylem parenchyma cells. Radial movement continued, usually intracellularly, until the fungus reached the exterior of the wood. The invaded ray cells proliferated and later collapsed, forming cavities into which the hyphae grew. The hyphae increased between the cambium and the bark, and there formed fungus mats consisting of a central core of hyphae surrounded by pseudoparenchymatous stroma. The mats enlarged as the hyphae resumed their thread-like structure, and formed a dense, interwoven network, containing scattered groups of large cells, one of which was greatly elongated. Each group of large cells was sheathed with a dense layer of small cells, thus resembling early stages of perithecial formation. In other areas of the stroma, cavities were formed into which conidiophores grew and produced endoconidia. Many large mats often formed on a single tree. Hyphae and tyloses also were found in the xylem vessels of infected oak roots. Hyphae invaded other cells of the root, including the cortex. The mode of hyphal migration was similar to that in the stem. Fungus mats were not detected in the roots. Spores were observed in the vessels of infected seedlings 4 days after inoculation, and both hyphae and tyloses formed within 18 days. Leaves on oaks infected late in the previous season wilted after the fungus invaded the new annual ring.

The development of the oak wilt fungus, *Ceratocystis fagacearum* (Bretz) Hunt, in the wood of northern pin oak, *Quercus ellipsoidalis* E. G. Hill, as well as the response of this host to fungus invasion, has received increasing attention. Beckman and Kuntz (1) traced the movement of poisons, dyes, and radioactive isotopes within and between both healthy and infected oaks. In healthy oaks, the upward movement in the trunks was rapid; downward movement was limited except in grafted roots. In infected trees, however, as further studied by Beckman et al (2), a sudden drop (90%) in the rate of movement in inoculated oaks occurred during a period 3–4 days preceding first permanent wilt symptoms. Struckmeyer et al (18) described the formation of tyloses and gums in the xylem vessels of infected oaks. These formations occluded the vessels, restricted internal water movement, and appeared to contribute to the development of foliage wilt. In histological studies of infected oak

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tissue. Struckmeyer and Kuntz (19) rarely observed mycelium in the vessels before or at the time of incipient wilt. Later, during severe wilt and defoliation, extensive mycelium developed in the xylem vessels. Fungus migration outward through the sapwood and initiation of mat formation were described. The present paper reports further studies of fungus activity within infected trees, certain host reactions to fungus invasion, and the initial stages of mat formation.

Both mycelium and spores have been reported in the sapwood of infected oaks as well as in felled logs and sawed boards. In earlier studies, Henry and Riker (11) recovered the fungus from naturally infected trees as deep as the two-year-old summer wood. Young (23) observed mycelium in some xylem vessels of leaf midribs, petioles, and twigs of infected red oaks, *Q. borealis* Michx. Mycelial development was generally sparse, with most hyphae growing longitudinally in the vessels. Radial distribution of the fungus was restricted to the outer ¼ in. of sapwood. Numerous conidia also were observed, and were suggested as a mechanism for rapid dissemination of the fungus throughout the tree in the transpiration stream. Jones and Bretz (12), however, were able to isolate the fungus from the tenth annual ring, or 32 mm inside the cambium, of infected red oaks that had died the previous summer. The authors concluded that the oak wilt fungus apparently was limited to the outer sapwood until shortly after foliar symptom expression, but that, by the time mats formed, the organism may have invaded any part of the sapwood. Englerth, Boyce, and Roth (8), in studying the longevity of the oak wilt fungus in felled red oaks, could isolate the fungus only in the outer sapwood of logs freshly cut, but from a depth of at least 1 in. in logs stored 6 weeks or in sawed boards after 14 days. The authors stated that the fungus had penetrated at least 1 in. during the air seasoning, but they did not describe the manner of penetration. Meanwhile, Fergus and Wharton (9) also observed tyloses in the vessels of twigs, branches, trunks, and roots of infected red oaks, *Q. rubra* L. Very few were detected in the roots. Sparse mycelium was found in the sapwood of the trunk and in a few roots, but no conidia were observed. The bark was not invaded by the fungus until mats had formed.

Critical differences in disease development in other oak species have been studied. Parmeter et al (14, 15), in studying infected bur oaks, *Q. macrocarpa* Michx., reported that symptomatic tyloses, which plugged the xylem vessels, were limited to a narrow, vertical sector of the bole and to vascularly connected branches. Such branches developed symptoms, and the oak wilt fungus was isolated from them. However, only a few hyphal fragments and conidia were detected in the vessels. Many trees failed to develop symptoms after the first season, and attempts to isolate the fungus from the next season's annual ring were negative. In other trees, recurring symptoms resulted from invasion of the new annual ring by the fungus and subsequent plugging of the vessels.

Stages in the life cycle of the fungus were unknown until Bretz (3, 4), Tiffany (20), and Wilson (21, 22) described the perithecial stage of the organism in culture. Curl, Stessel, and Zuckerman (5, 6) observed mats in the subcortical region on both the inner surface of the bark and the outer surface of the wood of wilt-killed trees. Perithecia have been found embedded in the mats, and their structure described (13, 16, 17).

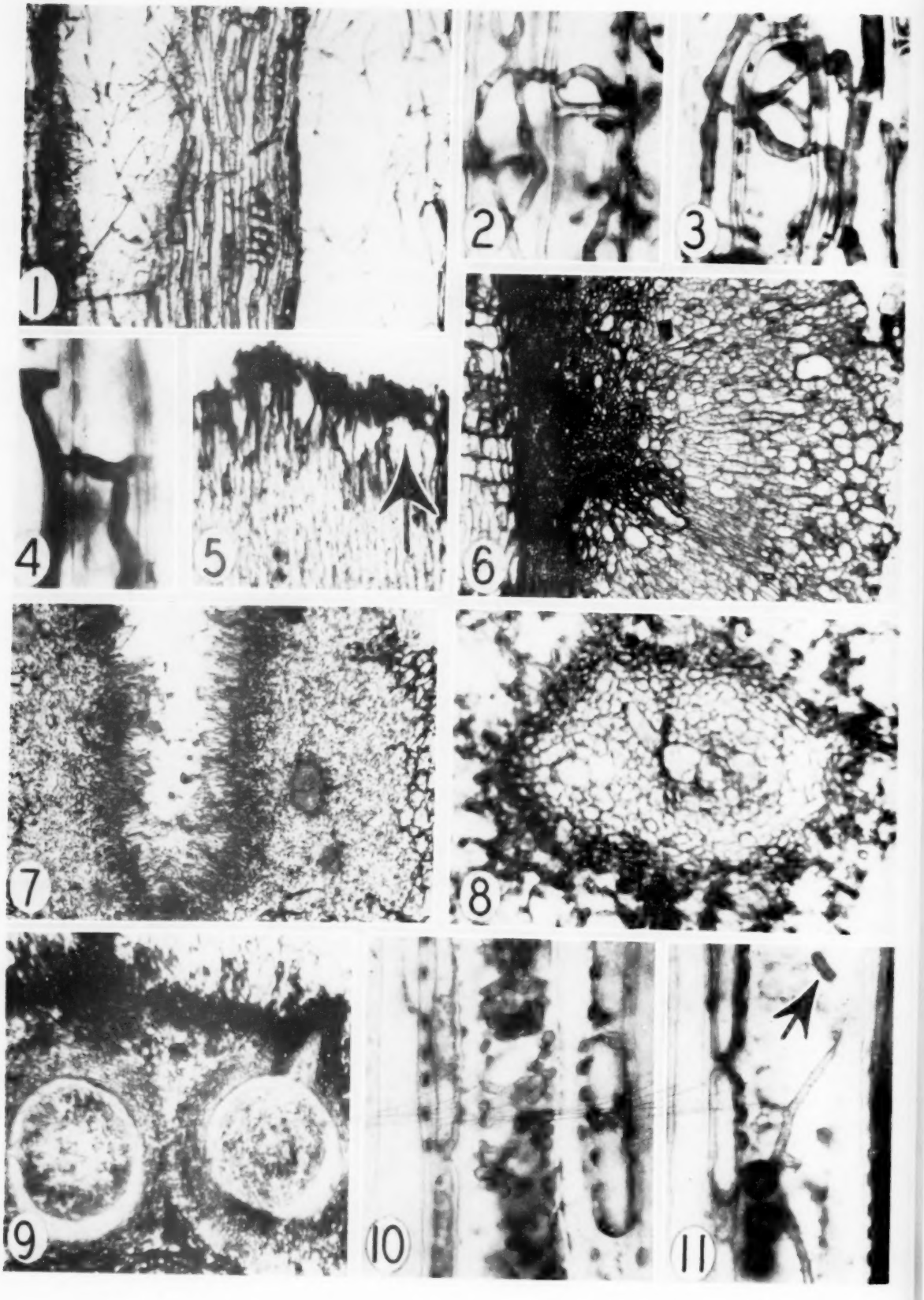
MATERIALS AND METHODS.—Various samples were prepared for histological study. Mats were collected in September, 1953, near Wisconsin Rapids, Wisconsin, from the branches and trunks of northern pin oaks that had wilted earlier the same summer. The mats ranged from 1 mm to 6 cm in length. The specimens were cut into small blocks prior to fixing. Samples of roots were taken in March, 1954, from similar trees that first showed wilt symptoms in July, 1953. The roots were about 1 in. in diameter. They were quartered before being placed in the fixing solution.

Two-year-old seedlings of live oak, *Q. virginiana* Mill. growing in a greenhouse were inoculated December 31, 1954, with spores introduced through wounds in the second and fourth internodes from the tip. The seedlings were grown at a temperature range of 20–37°C. Samples were taken through the inoculated region of these internodes 4, 18, 32, and 84 days later. Another group of seedlings was inoculated only at the fourth internode of the stem. Twelve weeks later, when wilt symptoms appeared in the crown, samples of the stem were taken at the inoculation point as well as 2 in. above and below.

A group of northern pin oaks inoculated August 6, 1954, developed foliage wilt by September 1. As occasionally happens, several of these trees partially refoliated the following spring. On May 13, 1955, samples were taken from these stems with active cambium. After the new foliage had wilted, sampling was repeated to determine whether the fungus had invaded the new growth ring.

Collected specimens of appropriate size were fixed in formalin-acetoalcohol. They were dehydrated with *n*-butyl alcohol and embedded in paraffin. Longitudinal sections 15–20 μ thick were cut on a sliding microtome and stained with safranin O and fast green FCF. The hyphae appeared light green and the nuclei red.

OBSERVATIONS.—*Stems of naturally infected trees.*—Hyphae of the causal fungus were rarely observed in the vessels or other tissue at the time of initial leaf wilt. Later, however, after most of the leaves had turned brown and dropped, extensive mycelial growth was found in the xylem vessels (Fig. 1). For example, the material collected in September, 1953, from trees that had wilted in midsummer had mycelium and tyloses in the vessels. Hyphae were abundant, particularly along the periphery of the xylem vessel wall.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On wood with tiny mats that were scarcely visible, hyphae were observed in the outer tissues of the wood also. The hyphae had reached the cambium by penetrating the bordered pit pairs of the xylem vessels into adjacent ray and xylem parenchyma cells. Thus, they made both lateral and vertical penetration through the stem (Fig. 2, 3). Similar penetration (Fig. 4) occurred in the root, as discussed later. Mycelial penetration was usually intracellular.

Proliferation of the ray parenchyma cells accompanied fungus invasion. As a result, masses of these cells formed in the outer sapwood near the cambium. To determine whether the ray cells proliferated before actual invasion of the fungus was difficult. However, cells through which hyphae had passed contained particles of dark-staining, granular material. By the time mycelium reached the cambium, many of the ray cells had collapsed and disintegrated, leaving masses of darkly stained material. The masses of collapsed ray cells formed cavities, which were occupied quickly by the mycelium (Fig. 5).

A mat of fungus mycelium was initiated as the hyphae grew between the cambium and the wood. Heavier staining of adjacent cell walls, collapse of surface cells, loosening of the bark, and an accumulation of fatlike droplets in ray parenchyma accompanied fungus activity. The enlarging mats consisted of hyphae organized into what was commonly designated a pseudoparenchymatous stroma. Within the stroma or mat was a central core of hyphae surrounded by a mass of enlarged, rounded cells (Fig. 6). This arrangement was characteristic of mats larger than 1×3 cm. As the mats continued to enlarge, the rounded, pseudoparenchymatous cells resumed a threadlike structure and continued to grow along the surface of the cambium. Within this network of hyphae, 2 or more large cells, surrounded by several dense layers of smaller cells, became conspicuous (Fig. 7). These structures, not unlike ascogonia (22), varied in size and were scattered throughout the mat. The apical cell frequently was elongated and curved, suggesting a trichogyne (Fig. 8).

The mature perithecium consisted of a globe, or sack-like structure, embedded in the mat, with a neck that protruded through the outer edge of the stroma and beyond (Fig. 9). Ascospores were formed within the rounded perithecium and released through the neck to the exterior. Young, developing perithecia, mature ones releasing ascospores, and old, empty ones occurred in the same stroma.

Simultaneously, cavities were formed in the stroma. Conidiophores from the hyphae surrounding these cavities grew into the cavity and formed endoconidia (Fig. 7). The endoconidia, when forming, appeared as a chain within the hyphal tip. The older, first-formed spores were released into the cavity.

Roots of naturally infected trees.—Roots from trees that showed wilt in July, 1953, were sampled early in March, 1954. At that time, some of the wood showed decomposition. Tyloses were abundant in the vessels, completely occluding them in some cases. In some sections hyphae were observed emerging from the vessels through the pits of the surrounding cells, as described for the stem (Fig. 4). Radial migration outward continued through the parenchyma cells. Cellular reactions within the roots were less than those in stems; there was no evidence of mat formation; the cambium was in contact with the bark in all samples examined. However, hyphae were found in the cortical cells, and some had penetrated between the layers of the periderm.

Stems of inoculated seedlings.—Two-year-old seedlings of live oak growing in the greenhouse were inoculated in the upper stem with the oak wilt fungus to determine how soon external and internal symptoms would appear after inoculation. Four days after inoculation, spores (probably some of those introduced) were observed within the vessels (Fig. 10). At this time, the vessels were still young, and cross walls had not yet dissolved. In some cases, incipient foliar wilt appeared 13 days after inoculation. Eighteen days after inoculation, hyphae and, occasionally, spores were found in the vessels above and below the junction of a wilted branch. Tyloses were protruding into the vessels; some tyloses were in an early stage of development, and others were large enough to occlude the vessel (Fig. 11). Twelve weeks after inoculation, when symptoms ranged from moderate to severe wilt, tyloses, hyphae, and spores were apparent in vessels at least 2 in. above and below the point of inoculation. By this time, tyloses had completely occluded the vessel. The hyphae migrated outward by growing through the bordered pits of vessels and then from cell to cell, as described previously.

Stems of refoliated trees.—A number of trees were inoculated August 6, 1954. These trees developed severe foliage wilt by September 1. In the following spring, several trees partially refoliated. Although leaf expansion continued for a time, foliage appeared sparse, stunted, and variously malformed. On May 13, 1955, samples of stem tissue showed mycelium and

FIG. 1–11. Photomicrographs of longitudinal sections of he stem of oak infected with the oak-wilt fungus.—Fig. 1. Mycelium in vessels. 800×.—Fig. 2, 3. Hyphae passing through bordered pit pairs of xylem in stem. Note the constriction in the hyphae that pass through the cell walls to the right. 800×.—Fig. 4. Hyphae passing through bordered pit pairs of xylem in root. 850×.—Fig. 5. Proliferated ray cells near the cambium have collapsed and formed cavities into which the mycelium grew (arrow). 120×.—Fig. 6. Early stage of mat formation showing stroma of pseudoparenchymatous cells. 165×.—Fig. 7. Part of a mat with endoconidia forming in the cavity, and different stages of perithecial formation in the stroma. 165×.—Fig. 8. Early stage of ascogonial development showing curved trichogyne. 800×.—Fig. 9. Mature perithecia in mat. 80×.—Fig. 10. Spores in xylem vessel 4 days after inoculation of 2-year-old live oak seedling. 800×.—Fig. 11. Tyloses, hyphae, and spore (arrow) in the vessel of two-year-old live oak seedling, 18 days after inoculation. 800×.

tyloses in the 1954 sapwood but not in the 2 tiers of newly formed springwood vessels. The xylem vessels were differentiated, but cross walls were still apparent. At that time, the fungus was readily isolated from the plugged 1954 annual ring, but not from the open 1955 springwood vessels. After May 20, no additional vessels formed, but the new springwood vessels were still open. On June 21, wood of the same trees was examined again. Meanwhile, leaf expansion had ceased and foliage wilt appeared. On this date, spores and mycelium were found in the newly formed xylem vessels; some of the vessels were occluded with tyloses. The oak wilt fungus was isolated from both the 1954 and the 1955 rings. In the stem, the hyphae traveled laterally via the ray cells, through the old matured cambium and into the newly formed cambium, and vertically through the pits of the xylem parenchyma. As the mycelium reached the phloem and cortical parenchyma, the mycelial strands appeared larger and more granular. Many of the ray cells contained deeply stained granules. Breakdown and obliteration of the cortical cells were common. Within the cavities resulting from this breakdown, masses of mycelium were observed.

Discussion.—Oak wilt is a vascular disease resulting from invasion of the water-conducting vessels by the oak wilt fungus. Sudden wilt, premature defoliation, and death of infected trees result, indicating a severe reaction. The first internal, structural symptom of oak wilt noted was the formation of tyloses and gums in the xylem vessels, prior to development of initial foliage wilt. It was not until trees were severely wilted that hyphae were observed in the xylem vessels of the stem. Their subsequent development and migration to other tissues gave some insight on fungus activity within the host that led to symptom development.

The reason for this delay in mycelial development is unknown. The difficulty of finding mycelium, even during the early stages of foliage wilt, suggests the importance of conidia in rapid internal distribution of the fungus. It also suggests that a study of spore physiology might clarify early host responses, such as rapid tylose and gum development.

Exit of the hyphae from the xylem vessels through the bordered pits of the ray parenchyma and xylem parenchyma preceded the initiation of fungus mats between the bark and the wood. The proliferation of the ray cells as the fungus grew toward the cambium apparently resulted from a stimulation by the organism, since the invaded ray cells contained granular particles, and these cells later collapsed. No doubt this proliferation and disintegration of the outermost parenchyma cells at the place of mat formation facilitated the loosening of the bark and, together with pressure exerted by the enlarging fungus mat, promoted bark cracking. Fergus and Wharton (10) recently demonstrated that the oak wilt fungus produced a growth-promoting substance as well as a pectin depolymerase in culture. Whether these or similar substances are active in infected trees is unknown.

Examination of the wood of northern pin oaks that wilted in 1954 but partially refoliated in 1955 demonstrated that the cambium remained alive and functional for a time even after severe foliar wilt. No immediate or direct "toxic" action on the cambium was noted. Foliar wilt and eventual death of the infected trees occurred only after actual fungus invasion of the current annual ring and subsequent plugging in the springwood vessels. No evidence was seen of diffusion of fungus metabolites from one ring to another.

In northern pin oaks the fungus appeared able to migrate readily from the 1954 infected annual ring into the newly formed 1955 springwood vessels. Such fungus penetration would account for the commonly observed (7) partial and abnormal refoliation of fall-infected trees the following spring.

Stages in mat formation and perithecial development on infected oak trees closely correspond to stages of development in culture (21).

Department of Horticulture
and
Department of Plant Pathology
University of Wisconsin
Madison, Wisconsin

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LEAFSPOT OF MARIGOLD, TAGETES ERECTA, CAUSED BY SEPTORIA TAGETICOLA N. SP.[1]

Winit Changsri and George F. Weber[2]

SUMMARY

A leafspot disease of *Tagetes erecta*, caused by **Septoria tageticola** n. sp., is characterized by oval to irregular, smoky-gray to black spots on the leaflets, speckled with minute black pycnidia. In nature the disease first appears on the lower leaves and advances upward. It also develops on the younger branches, peduncles, bracts, and seed. Inoculation studies show penetration of the host through the stomata and the production of viable pycnidiospores after an incubation period of 4–5 days. Horticultural varieties of the African marigold, *Tagetes erecta* L., are very susceptible, whereas varieties of the French marigold, *T. patula* L., are resistant to immune.

Introduction.—The commonly grown ornamental marigolds, characterized by Bailey (1) and Small (2), are horticultural varieties of *Tagetes erecta*, the African type, and of *T. patula*, the French type. Weiss (4) reported many parasites causing diseases of marigolds, but no reports of *Septoria* spp. on any *Tagetes* spp. have been found in the literature.

Previous collections of the disease, made by R. K. Voorhees in 1936, and by G. F. Weber in 1955, are in the Florida Agricultural Experiment Station Herbarium, designated as *Septoria* sp. A preliminary report of the disease was made by the writers (3) in 1957.

This disease has been found only on the marigold species *Tagetes erecta* and *T. patula*, the former being more susceptible than the latter. On susceptible plants, the severity of the disease was closely correlated with climatic conditions.

This leafspot was found in several widely separated locations in Alachua County, and a limited survey of the vicinity within 150 miles of Gainesville revealed the disease on the African varieties almost without exception.

The variations of the disease were observed during the summer of 1957. Symptoms were so severe that the blooming period was shortened by premature defoliation and early death of the plants. A survey in this vicinity showed the disease in 47 plantings of the African type marigold. During the entire summer no plantings were found free of the disease. Severe outbreaks depended largely on high temperatures, rainy periods, and continuing periods of high humidity. Primary infection, apparently from infected seed, was observed in early spring on volunteer plants from a previous planting.

Under favorable conditions the fungus advanced extensively in the host tissue and produced an abundance of pycnidia and pycnidiospores, which were scattered by blown leaf fragments, rain, and dew. Secondary infection continued to spread rapidly.

The disease is considered the most important factor in shortening the longevity of the marigold as a flowering ornamental in this part of Florida. Plants grown before July usually escape the disease because they develop during dry weather.

Symptomatology.—Plants in any stage of develop-

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[2] Respectively, graduate student supported by the Thai government, and Professor of Plant Pathology, College of Agriculture, University of Florida. The writers are indebted to Dr. Joseph Brunet, Head of the Department of Foreign Languages, University of Florida, for preparation of the Latin diagnosis.

ment were susceptible to infection by the fungus. When flecks were first observed on the leaflets, they were a darker green than the surrounding tissue. Under favorable conditions they enlarged rapidly, becoming smoky-gray to near-black, oval to irregular in shape, and speckled with minute black pycnidia (Fig. 1-a,b,c). As the disease developed the leaflets became curled and twisted, but remained attached to the leaf rachis.

Lesions on the petioles, stems, and peduncles, less conspicuous than on the leaflets, formed elongate, narrow, discolored areas in which black pycnidia

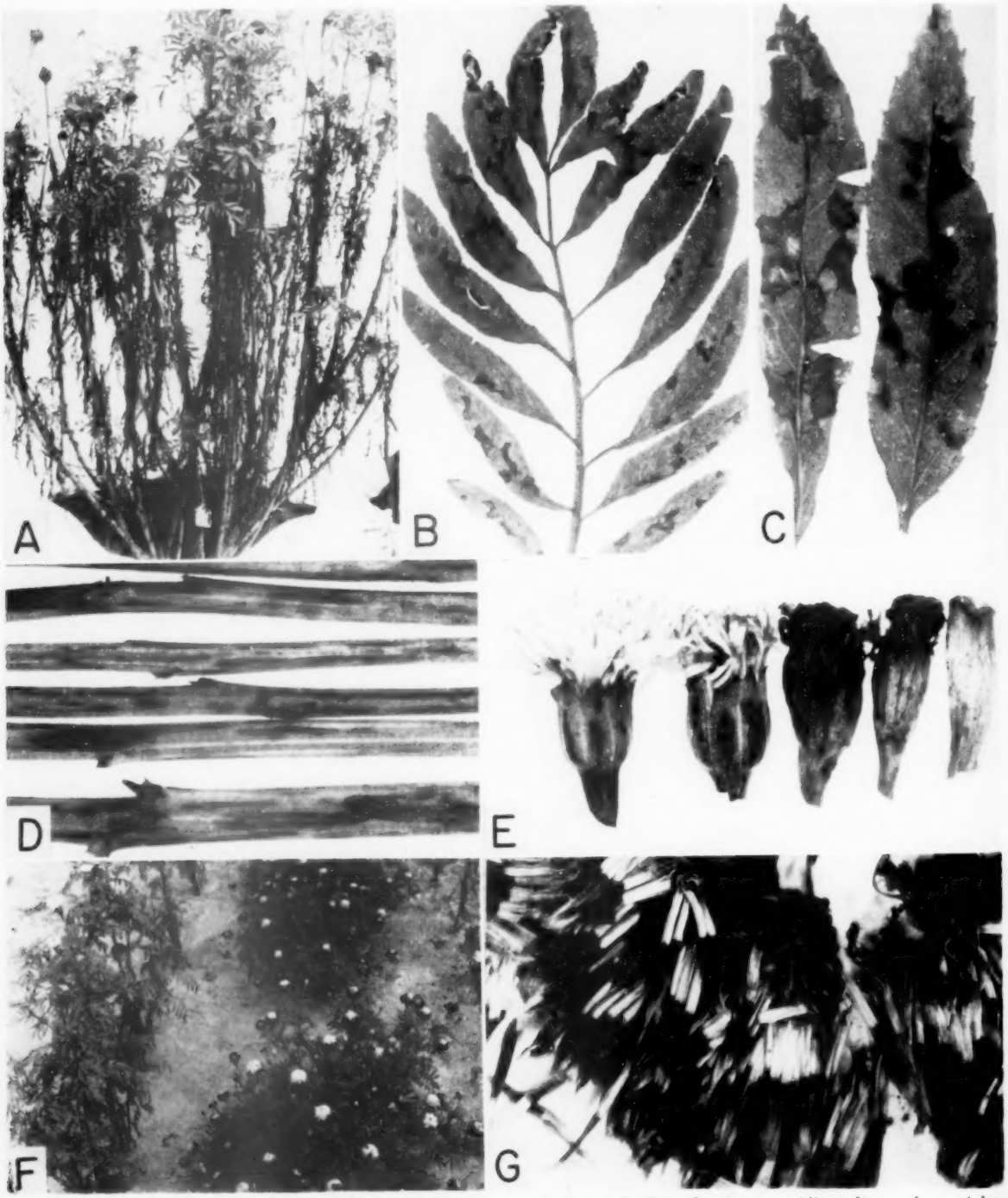

FIG. 1. Leafspot disease a) on a susceptible marigold; b) on a leaf; c) leaflets showing pycnidia; d) on the petioles; e) on the involucral bracts; f) showing susceptible *Tagetes erecta* (left) and resistant *Tagetes patula*; g) pycnidia on seed.

were usually seen (Fig. 1-d,e). Their size was limited mostly by the diameter of the host part. On the involucral bracts, the blackish lesions were usually longer than wide, and contrasted with the green of the surrounding tissue.

Seeds that developed in heads enclosed by diseased bracts also became infected (Fig. 1-g). Pycnidia were found on the seed near the point of attachment.

The fungus grew slowly on potato-dextrose agar at 4–28°C. with optimum rate for mycelium and pycnidiospore production at 20–24°C.

Scanty growth developed at pH 3.0; optimum growth appeared at pH 4.0–6.0. Development of the fungus in alkaline media slowed as alkalinity increased, although growth was fair at pH 9.1. Pycnidia developed plentifully at pH 4.1, though pycnidiospore formation was poor. Because the growth was exceedingly slow, the diameter of colonies at optimum rate was 2.2–2.6 cm at 30 days.

Growth was better on potato-dextrose agar than on celery, onion, marigold, V-8, Difco bean pod, Difco Lima bean, Difco corn meal, and Difco prune agar.

Pycnidiospores germinated better on potato-dextrose agar than in distilled water. They swelled, became vacuolate, and deeply constricted at the septa, and increased in length (Fig. 2-a). The terminal germ tubes were well developed at 18 hours. The mycelium branched and extended rapidly, becoming dense, and light-brown jelly-like pycnidia were initiated within 4 days (Fig. 3-a). Secondary and tertiary pycnidia developed rapidly at the same time, varying from light tan to near black. In many instances pycnidiospores developed within 9 days.

MATERIALS AND METHODS.—Sixteen varieties of the African and French marigolds were grown both in the greenhouse and outside. Throughout the study we used pure cultures of the fungus from many isolations of naturally infected marigold plants from widely separated areas in Florida.

Cultures of *Septoria tageticola* were obtained from infected leaves, stems, and bracts. Conidia produced by the several cultures originating from these various isolations were used as inoculum. Various ways were tried of inoculating healthy plants with the fungus. All inoculated plants were left 48 hours in a moist chamber and removed to the greenhouse bench. Five days after inoculation, light-brown diseased spots began to appear on the leaflets. Inoculations in the greenhouse included plants incubated 2 days under a cheesecloth cover with a mist atomizer suspended overhead. Other inoculated plants were left uncovered, with no humidifier. Plants growing in a field plot were inoculated by atomizing spore suspensions onto the foliage after the plants and soil around them had been thoroughly wetted. The amount of disease resulting was very similar in all tests. All inoculations were made in the evening, when relative humidity was high. Black pycnidia usually developed in 9–10 days.

In varietal susceptibility studies, a series of 3 inoculations were made in the variety field plot on July 29, August 5, and August 18, 1957. One or more plants of each variety were reserved as checks. The horticultural varieties of *Tagetes erecta* were susceptible, and the horticultural varieties of *T. patula* were resistant (Fig. 1-f).

The mycelium of the fungus, as observed in culture soon after germination, was hyaline, 4–6 μ in diameter, and became septate and many-branched. It became gray with age, and eventually almost black.

The pycnidia were spherical and developed on both surfaces of the brown lesions, though they were more numerous on the lower leaf surface. Detailed studies were made from fresh host tissue left in a 1:1 solution of absolute alcohol and glacial acetic acid until the

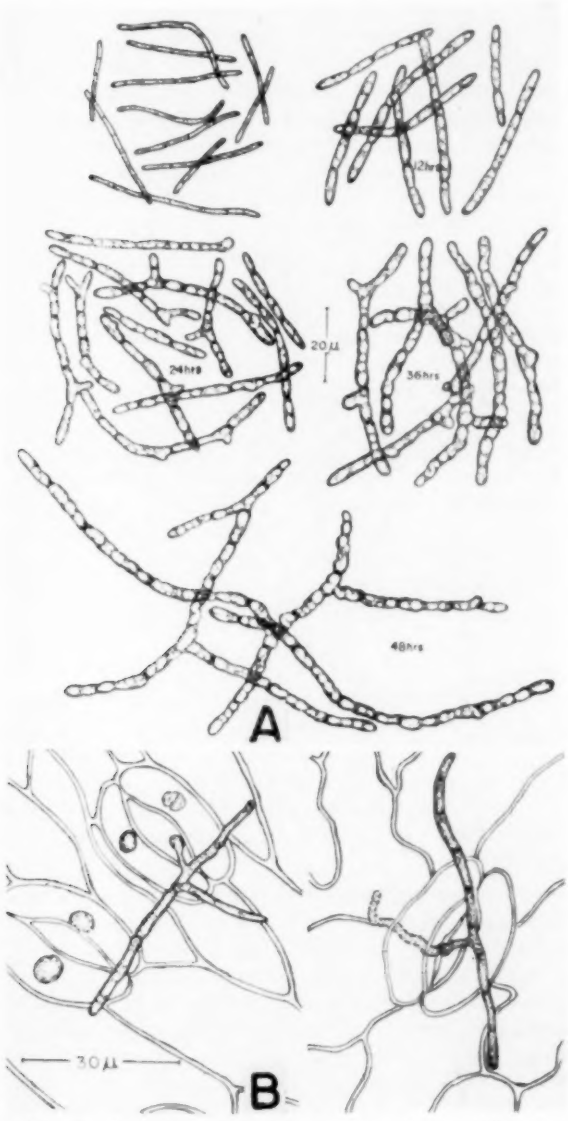

FIG. 2. *Septoria tageticola* showing a) pycnidiospores and stages of germination; b) camera lucida drawing illustrating stomatal penetration.

chlorophyll was removed. The tissue was then gently boiled a few minutes in lactophenol to shorten the time of clearing, cooled and left in a saturated aqueous solution of chloral hydrate until clear, and mounted and ringed on a glass slide.

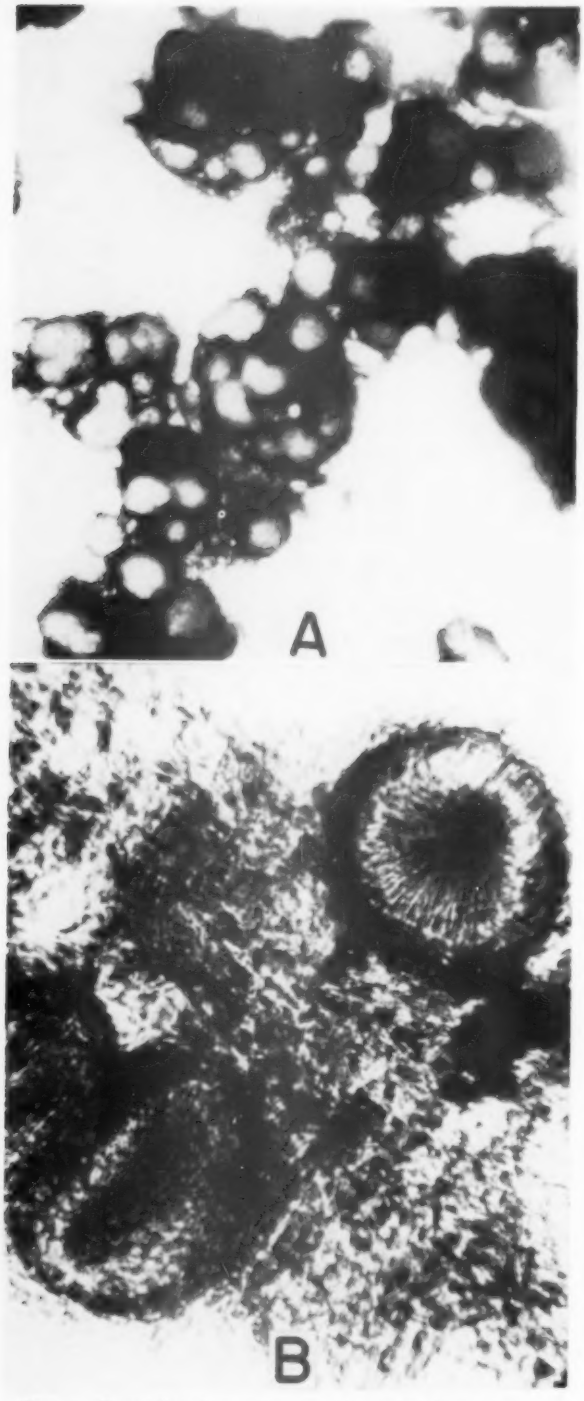

FIG. 3. Pycnidial formation on a) potato-dextrose agar; and b) on host.

A *Tagetes erecta* variety, Yellow Supreme, was inoculated with a spore suspension of the fungus from potato-dextrose agar and incubated in a humid atmosphere. Detached leaves were also inoculated, and incubated in moist chambers. Inoculated areas on leaves were indicated with India-ink marks. After an initial 12-hour incubation, some leaves were excised each 6 hours for 72 hours. Samples were cleared, stained, dehydrated, and mounted permanently on glass slides for study.

Examination of the prepared sections showed that, during the first 18 hours of incubation, the pycnidiospores germinated from the terminal cells, and secondarily from the remaining cells. Penetration had taken place through the stomata of samples collected 36 hours after inoculation (Fig. 2-b). The stomata were penetrated, regardless of whether open or closed, and no direct penetration through the epidermis was observed. Germ tubes that penetrated the host through the stomata usually showed the formation of an appressorium at the point of entry. Germ tubes were not always attracted to the stomata; in many instances they grew across the opening. The appressoria were 1.5–3.0 μ in diameter. At first the germ tubes became slightly enlarged at the tip, and later formed an appressorium and a penetrating hypha. The hyphae were located in the intercellular spaces throughout the parenchyma cells. Pycnidial initials appeared as aggregates of hyphae in the substomatal chambers, where the mature structures were rapidly formed. Some appeared to be formed deeper in the host tissue (Fig. 3-b).

The fungus was classified in the genus Septoria because of the color and shape of pycnidia and pycnidiospores. No sexual stage has been observed. A report of a disease of marigolds caused by a *Septoria* sp. has not been found. A number of *Septoria* spp. were reported on plants of the family Compositae that grow in Florida; however, none of the descriptions were considered similar to *Septoria tageticola*, except possibly *Septoria chrysanthemi*, which has slightly larger pycnidia and larger spores. Cross-inoculations, however, showed that *S. tageticola* was not pathogenic on chrysanthemums and that the *Septoria* sp. found on chrysanthemums did not infect marigolds. It was concluded that the fungus is an undescribed species, and the following description is presented:

Latin description.—Pycnidia globosa maculicolosa, aggregata, caulicolosa, phyllogena, amphigena saepius hypophylla, subepidermalis dein de erumpentia, fulva dein nigrantis, 42–140 μ in diametro: primum, parvo-ostiolata, in maturitate late aperta, conidiophora hyaline, subulater 3–6×1–2 μ: conidia singulatim orienda, hyalina, filiformia, continuosa dein 5 septate, recta, 18–57 ×1–1.5 μ in cirros emergentia.

In caulibus, foliis Tagetes spp., Gainesville, Florida.

Description.—Pycnidia round, aggregated in spots on the stems and the upper leaf surfaces, more com-

mon on the lower surface; subepidermal, becoming erumpent, brown becoming black; 42–140 μ in diameter; at first a small circular ostiole becomes widely open, 14–70 μ at maturity, conidiophores hyaline, subulate, 3–6×1–2 μ; conidia borne singly, hyaline, thread-like, straight, continuous, becoming 5-septate, 18–57×1–1.5 μ, emerging in tendrils.

On stems and foliage of *Tagetes* spp., Gainesville, Florida.

Type material number F 46421, Herbarium, University of Florida.

PLANT PATHOLOGY DEPARTMENT
UNIVERSITY OF FLORIDA
GAINESVILLE, FLORIDA

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SOME FACTORS THAT INFLUENCE DEVELOPMENT OF DWARF BUNT IN WINTER WHEAT[1]

L. J. Tyler and N. F. Jensen[2]

SUMMARY

Coverings of straw or snow on plants during the winter months, when wheat is relatively dormant, induced development of dwarf bunt, caused by *Tilletia contraversa*, much in excess of that in plants not covered. Coverings of straw were more effective than snow. Light coverings of straw applied immediately after sowing wheat did not induce development of the disease; in fact, such coverings remaining on the plots during autumn and winter tended to depress the disease. The means by which winter coverings of straw or snow induce development of dwarf bunt are unknown. Probably winter cover minimizes the effects of certain climatological factors when they become unfavorable to the pathogen and helps to create an environment favorable for attack by its stabilizing effects on soil moisture, temperature, and, perhaps, light at the ground level. Straw cover has been used successfully for inducing development of dwarf bunt of wheat in a program designed to identify resistant selections. Incidences of dwarf bunt were higher in wheat plantings made at 1-in. depth than in those made at 2-in. depth. The disease-producing potential of infested soil was found to be related to the load of inoculum. Moreover, it was found that the disease-producing potential of soil-harbored inoculum may diminish considerably within a 2-month period if the infested soil is intermittently wetted by rain.

Observations in commercial wheat fields in New York, beginning in 1945, led to the hypothesis that winter cover, such as deposits of drifted snow, induced development of dwarf bunt in winter wheat. Experiments revealed that straw coverings applied about the time wheat becomes winter dormant induce development of dwarf bunt in plants growing in soil infested by *Tilletia contraversa* Kühn (18). Since dormancy is manifested by the spores of this pathogen, little if any infection can be obtained by inoculating seed. Infection of wheat can be obtained, however, by planting seed in soil infested by the pathogen, but the resulting prevalence of disease may be too low to further the objectives of an experiment. For example, tests of wheat selections for reaction to *T. contraversa* have frequently been rendered futile by the uncertainty that attends the development of dwarf bunt in plants grown outdoors in infested soil. It seemed worthwhile, therefore, to investigate further the possibility of using straw cover to induce dwarf bunt in wheat. Continued investigation of this phenomenon has produced results in each of 3 years similar to those first reported in 1953 (18). During this period the straw-cover method has made possible steady progress in the search for dwarf-bunt-resistant selections of wheat for New York (11).

This paper presents results of experiments performed to provide additional information on how the development of dwarf bunt in winter wheat is affected by straw cover, the timing of application and removal of straw cover, soil moisture, methods of seeding, weathering of spores in the soil, and methods of applying infested soil in the process of inoculation.

MATERIALS AND METHODS.—Inoculum of *T. contraversa* was collected from diseased wheat on New York farms and from diseased wheat that developed in the dwarf-bunt nursery. Heads affected by dwarf bunt were collected in late July or early August, dried, and stored at normal indoor temperatures until prepared for use in autumn. The inoculum was usually prepared by grinding dried diseased wheat heads coarsely in a meat chopper. Measurements show that the smut sori in a head of diseased wheat compose, on the average, about 50 per cent of its total weight.

[1] Accepted for publication June 2, 1958.
[2] Professors of Plant Pathology and Plant Breeding, respectively.

Ground inoculum was mixed with moderately dry field soil, and the infested soil was stored in earthen-floored outdoor bins at the experimental nursery. During storage the infested soil was exposed to, or protected from, rain as desired. Soil and inoculum were mixed as follows: Two bushels of soil were spread in a layer on the earthen floor of a 4 × 8-ft. outdoor bin. Friable soil with low moisture content proved a satisfactory medium in which inoculum could be distributed with minimum difficulty. Inoculum in desired quantities was uniformly spread on the soil layer and raked in. Succeeding measured quantities of soil were spread on the previously mixed layer, and inoculum was added and mixed as before until the infested soil reached a depth of 9–10 in. in the bin.

Inoculum used in experiments performed in 1954 was subjected to weathering factors by 2 methods. Inoculum designated "1953 one-year weathered" consisted of sori collected in 1953, placed in pure glass-cloth bags (about 2 teaspoonsful of sori per bag), and cached on the soil surface outdoors for about 1 year, exposed to the elements of weather and edaphic factors (17). Spores so treated were mixed with soil on September 24, 1954, and kept protected from rain in an outdoor bin. Inoculum designated "1954 weathered" consisted of coarsely ground inoculum collected in 1954; it was mixed with soil at 100 g/bu. and exposed in outdoor bins to rain. A similarly prepared batch, referred to as "1954 not weathered," was covered to protect it from rain. The 2 latter batches were prepared on August 9, 1954, and used in early October of that year. Infested soil used in 1955 and 1956 was prepared as described at the beginning of this section, and protected from rain until used.

Winter wheat seed (Cornell 595) was sown in open rows 4–5 ft. long, 3 rows per plot, and the plots planted in triplicate. Equal weighed quantities of either formaldehyde-treated or hot-water-treated seed were planted per row. Two methods of planting were used. One method, called "regular," consisted of planting seed at either 1- or 2-in. depths in rows opened in loose, fitted soil at normal ground level. The second method, called "basin," consisted of planting seed at 2-in. depths in rows in depressions made by removing loose soil so that the bottom of the depression was firm and resembled a wide dead-furrow. About 1 gal. of infested soil was used to cover seed in each row. The infested soil was packed by walking on it after placing it in the seeded row.

Coverings of oat straw were applied at 2 rates: "heavy" applications were about 22 lb. per 100 sq. ft. of field-plot surface; "light" applications were about 15 lb. per 100 sq. ft. Considerable care was taken to distribute the straw uniformly on the plots, after which it was battened down with twine.

An effort was made to obtain extra-heavy snow cover on some plots by constructing a 20-in.-high snow fence along the prevailing windward side of the plots.

Data were obtained about the time the wheat began to ripen. The percentages in the tables were based on counts of total and smutted tillers.

Results.—Data in Table 1 show that incidences of dwarf bunt were greater in plots provided with winter coverings than in plots not covered. Straw was much

Table 1.—*Effect of coverings of straw and of snow on incidence of dwarf bunt in winter wheat seeded on different dates and covered with soil infested with differently weathered spors of Tilletia contraversa, 1954*

Seeding date	Year spores collected, and treatment[c]	Average per cent of tillers affected							
		Straw applications[a]							
		Dec. 3, 1954		Dec. 27, 1954					
		Heavy	Light	Heavy	Light	Snow cover[b]			No cover
Oct. 1	1953,[d] 1-year weathered	11.4	23.6	20.4	22.9	9.1	5.4	2.9	3.1
	1954,[e] not weathered	82.2	85.9	89.7	89.0	21.3	36.6	28.9	10.7
	1954,[e] weathered	61.9	28.4	71.5	30.9	10.5	19.6	18.2	8.7
Oct. 8	1953, 1-year weathered	14.3	6.5	7.5	15.3	3.3	0.5	1.1	0.0
	1954, not weathered	50.0	38.7	36.5	27.6	25.3	13.2	22.1	8.8
	1954, weathered	46.8	33.4	25.8	34.6	12.6	9.9	9.2	7.1

[a] Applications of straw: "heavy" = 22 lb., and "light" = 15 lb./100 sq. ft. of plot surface. Straw was removed from plots Apr. 2, 1955.

[b] Snow cover obtained by placing snow fence along windward side of plots.

[c] Inoculum collected in 1953 was weathered outdoors for about 1 year in glass-cloth bags on the ground surface. Inoculum collected in 1954 was coarsely ground, mixed with soil, and stored 10 in. deep in outdoor bins; one part, called "1954 weathered," was exposed to rain, another, called "1954 not weathered," was protected from rain.

[d] 14 g of spores per bu. of soil; infested soil prepared 6–14 days before used to cover seed.

[e] 100 g of ground inoculum per bu. of soil; infested soil prepared 52–60 days before used to cover seed.

more effective than snow in inducing dwarf bunt. The plots were bare of snow for various periods, which may have reduced the effectiveness of the snow cover. In fact, from December 3 to December 27, 1954, snow depth was 2–5 in. in the open, and 4–10 in. on plots with snow fences. Daytime temperatures of 37–50°F the last 5 days of December removed the snow, and the plots were bare until January 13, 1955. Then, until about February 21, the snow depth was 2–3 in. on unfenced plots and 4–7 in. on fenced plots. Warmer daytime temperatures began on February 20, and within 2 days all snow had disappeared from unfenced plots, and melted to 2–3 in. on fenced plots. These deposits melted away by February 28, and after this date there were no appreciable deposits of snow on the plots.

Data in Table 1 show that the over-all incidence of dwarf bunt was higher in some plots that received "heavy" applications of straw than in some that received "light" applications, but the differences are probably not significant. The difference in dates of straw application (December 3 and 27) appears not to have affected the incidences of dwarf bunt; the intervening period was apparently not critical for establishment of the pathogen in wheat plants in the 1954–55 experiment.

The incidence of dwarf bunt was definitely influenced by the 1-week difference in planting date (Table 1). With few exceptions, and regardless of cover treatments or inoculum treatments, incidences were higher in October 1 plantings than in October 8 plantings.

Finally, the data in Table 1 clearly show that the incidence of dwarf bunt was influenced by the amount of inoculum in the soil and by the weathering of the infested soil before it was used to cover seed. Soil infested with 100 g/bu. produced 3–10 times as much dwarf bunt as did soil infested with 14 g/bu. The effectiveness of the 14-g dosage was probably also reduced by the one-year weathering treatment to which the spores were subjected. Soil infested with inoculum and stored 52–60 days was definitely more effective if protected from rain instead of being exposed. These differences are shown even by the data obtained from check plots. It is clear that the weather and factors in the soil environment may together effectively reduce the disease-producing potential of soil-borne inoculum, even within a 2-month period.

Higher incidences of dwarf bunt have been observed in wheat in dead-furrows than in nearby plants. Therefore, an experiment was performed to obtain more information about this phenomenon. Additional information was sought about the effect of varying the inoculum load in soil. Wheat was sown in "basin" plantings and in "regular" plantings. Both types received winter coverings of straw, and plots of both types were left uncovered, as checks. Data show that the incidences of dwarf bunt were consistently higher in the "basin" plantings than in the "regular" plantings—especially in straw-covered plots, though the trend was similar in uncovered plots (Table 2).

TABLE 2.—*Effect of straw cover, planting treatments, and spore load of the soil on the incidence of dwarf bunt in winter wheat, 1955*

Inoculum/bu. of covering soil (g)[a]	Per cent of tillers affected[b]							
	Plots covered with straw[c]				No cover			
	Basin[d]		Regular[d]		Basin[d]		Regular[d]	
33	14.0		23.8		7.0		1.8	
	45.2	28.0	26.4	25.0	5.5	5.2	4.8	3.2
	14.2		25.2		2.6		4.2	
66	42.8		45.9		7.0		8.2	
	71.0	65.3	43.0	41.2	13.0	9.0	9.7	7.5
	72.2		36.9		6.8		8.7	
100	48.1		31.8		21.0		8.5	
	54.2	47.4	31.2	35.2	17.2	15.7	7.8	6.9
	42.5		40.5		7.1		4.5	

[a] Infested soil prepared Aug. 20, 1955, and protected from rain until it was used to cover seed, Oct. 3, 1955.

[b] Percentage for each of 3 plots, and average of plots.

[c] "Heavy" applications of straw on Dec. 2, 1955; removed Apr. 1, 1956.

[d] Seeds planted at about 2-in. depth and covered with infested soil. "Basin" plots consisted of plantings of seed in depressions in the ground surface that resembled dead-furrows. "Regular" plots consisted of plantings in soil at normal ground levels.

The load of inoculum in the soil was also of significance. Soil dosed with 66 g. of inoculum per bu. produced about twice the incidence of dwarf bunt as soil containing 33 g/bu. With one exception, soil dosed with 100 g of inoculum per bu. failed to equal or surpass the soil with 66 g, which suggests that little if anything is to be gained by doses of inoculum greater than 66 g.

The repeated demonstrations that winter coverings of straw induce development of dwarf bunt in wheat under New York conditions led to experiments in 1956 to determine the opportune times for applying and removing straw. Methods of planting seed and of applying infested soil were studied in conjunction with straw treatments. Materials and procedures were as already described except that straw applications on October 9, 1956, were made before the seedlings emerged, and were necessarily "light" to permit young plants to push through the straw without injury. The quantity of straw applied did not completely cover, and exclude light from, the ground surface, though it was sufficient to retard evaporation of moisture from the soil. Straw applied before seedlings emerged did not increase the incidence of dwarf bunt above that in uncovered check plots (Table 3). In fact, the incidences of dwarf bunt were consistently lower than those of the check plots unless this early straw covering was removed about 1 month after application. Then the incidences were about equal to those in the check plots.

In plots covered with straw on December 1, 1956, the incidence of dwarf bunt ranged from 16 to 41 per cent (Table 3), 10–15 times as great as in the check plots. Removal of straw from some plots on

January 4, from others on February 25, and from others on April 19, 1957, resulted in average percentages of dwarf bunt of 23.4, 31.0, and 24.0, respectively. Average incidences in the second group of plots were consistently higher than in the other 2 groups.

Some differences in dwarf bunt development appear to be correlated with methods of planting and of applying infested soil (Table 3). Since not all plantings could be made the same day, direct comparison of each treatment with each of the others is not possible. Comparison of the results from the 2 "heavy" straw-covered plantings on October 5 show that the average incidence of dwarf bunt was consistently higher in plantings 1 in. deep than in those 2 in. deep. Direct comparison of the plantings of October 6 and 8 seems justifiable because inoculum was applied to the plots on the same date and there was only a 2-day difference in date of seeding. Development of dwarf bunt appears distinctly better in the October 6 planting, though the difference may not be significant. Results from this planting indicate that inoculum does not need to be placed in contact with the seed at planting time; in this treatment, seed was first covered 1 in. deep with non-infested soil, which was followed 2 days later with a top dressing of infested soil. Comparison of the data for the October 3 plantings reveals small differences, mostly in favor of the non-packed soil treatment, but these differences are considered insignificant.

Discussion.—Coverings of straw applied at the proper time on field plots of winter wheat growing in infested soil have consistently induced much more dwarf bunt than that which developed in wheat not covered. Straw applications in December have repeatedly been effective in inducing disease develop-

Table 3. *Effects of applying and of removing straw at different dates, and of different methods of planting on the incidence of dwarf bunt in winter wheat, 1956*

Planting date	Method of planting: Depth	Method of planting: How covered	Per cent of tillers affected[b]: Straw applied Dec. 1; removed as indicated[c]: Jan. 4 1957	Feb. 25 1957	Apr. 19 1957	No straw (check)	Straw applied Oct. 9; removed as indicated[d]: Not removed	Nov. 5 1956	Dec. 3 1956
Oct. 5	2″	Infested soil[a]	13.4	22.0	17.3	1.6	1.0	3.4	0.2
			18.0	12.0	10.0	0.0	0.2	0.0	0.5
			17.5	28.8	23.0	3.1	0.0	5.0	3.0
		Average	16.7	21.0	16.2	1.6	0.4	3.0	1.0
Oct. 5	1″	Infested soil	21.0	30.2	26.0	0.0	0.0	1.0	0.0
			22.4	40.4	41.0	3.4	0.0	2.0	1.0
			17.0	25.0	20.0	3.2	1.0	5.0	0.0
		Average	20.0	32.3	30.0	2.0	0.3	2.4	0.3
Oct. 6	1″	Field soil, then 1 in. infested soil on Oct. 8	15.0	37.0	16.0	0.0	0.0	0.0	0.0
			49.0	39.2	32.1	0.0	1.0	0.0	0.0
			29.0	44.0	24.0	1.7	0.0	4.4	0.0
		Average	34.0	41.0	25.0	0.5	0.3	1.4	0.0
Oct. 8	2″	Basin;[e] infested soil	23.4	31.0	27.0	3.3	0.0	2.0	2.0
			29.0	19.2	24.2	2.1	0.3	4.2	0.0
			25.0	34.3	25.0	6.0	0.0	4.0	1.0
		Average	26.0	30.0	26.0	4.0	0.1	3.0	1.0
Oct. 3	2″	Infested soil, packed	17.0	31.0	15.0	1.6	0.0	0.0	0.0
			19.0	23.1	22.0	1.2	0.0	0.0	0.0
			38.0	31.1	27.0	2.3	2.3	0.4	1.0
		Average	26.4	29.5	21.0	2.0	0.8	0.2	0.3
Oct. 3	2″	Infested soil, not packed	17.3	18.0	20.3	1.1	0.8	1.6	0.0
			13.3	29.2	14.0	2.7	2.0	4.0	2.0
			19.4	44.0	27.0	4.0	0.0	4.0	0.6
		Average	17.3	31.4	21.0	3.0	1.0	3.1	0.8
		Average	23.4	31.0	24.0	2.2	0.4	2.2	0.5

[a] Infested soil prepared on Sept. 24, 1956; 60 g of ground inoculum added/bu. of soil, and the infested soil protected from rain until used.
[b] Percentage for each of 3 plots and the average.
[c] "Heavy" application of straw.
[d] "Light" application of straw.
[e] "Basin" plots consisted of plantings of seed in depressions in the ground surface that resembled dead-furrows.

ment. In contrast, coverings of straw applied immediately after seeding, in early October, have not induced development of dwarf bunt; prevalences of the disease in plots of the latter type have been similar to those in plots of wheat not covered with straw. Dewey (4) compared the effects of straw coverings applied on wheat in December with the effects of coverings applied in March; the former induced development of dwarf bunt, but the latter had no appreciable influence on the disease. Experience shows so far, therefore, that early winter applications of straw favor development of dwarf bunt in winter wheat, whereas early autumn and early spring applications have little or no effect.

Deposits of snow caused to lodge on winter wheat also induced development of dwarf bunt, but the disease was not as prevalent as that induced by December applications of straw (Table 1). The results support the belief, expressed earlier by the writers (18), that heavy and persistent deposits of snow tend to induce development of dwarf bunt. Sachs noted in Germany, in 1954, that dwarf bunt occurred only in sheltered locations with persistent snow cover (16).

How long straw cover is necessary to induce dwarf bunt is not definitely known. Certain data herein show that straw coverings in place during the winter months (December–February) induce disease development. Dewey concluded that "straw cover produces its greatest effect during the winter months when plants are relatively dormant" (4). Conceivably, the essential period might vary from year to year with variations in the weather. In plots at Ithaca, New York, where selected lines of wheat are tested for reaction to *T. contraversa*, the straw is left on the plants until about April 1; this procedure has resulted in satisfactory screening tests despite yearly variations in the weather.

Winter coverings of straw retard, and inhibit to some extent, the growth of winter wheat if allowed to remain on the plants until late winter or early spring (18). Similar effects on wheat have been noted where heavy deposits of snow were lodged. Since high prevalence of dwarf-bunted tillers has been consistently associated with retarded and inhibited responses of plants to straw and heavy snow cover, it appeared that such responses might have predisposed the plants to the disease. This apparent relationship between growth of wheat and prevalence of dwarf bunt was investigated by Dewey (4). He retarded the growth of wheat plants by treating some with maleic hydrazide, clipping the tops of others, and covering others with straw. The retarding effects of maleic hydrazide and clipping treatments on the growth of wheat did not induce development of dwarf bunt; in fact, one of the maleic hydrazide treatments appeared to suppress the disease. In contrast, coverings of straw induced up to 33 per cent dwarf-bunted tillers. Dewey stated that the "treatments used in an effort to simulate the growth-retarding effects of straw cover might differ in their mode of action from that of straw and that conclusions about the role that retarded growth plays in dwarf bunt development cannot be made on the basis of these [his] results."

Data obtained over a period of 4 years (not tabulated in this paper) show that straw cover applied on plants in December and not removed until early April results in about 16 per cent fewer tillers in covered plots than in non-covered plots. Straw reduces tillering in wheat, both in the presence and in the absence of the pathogen in the soil. Straw cover also suppresses the height of wheat culms and lengthens the period needed for maturation. This suggests that protective cover delays development of tiller initials or slows their rate of development, or both; consequently, protective cover may cause the plants to provide tissues susceptible to attack over a longer period. Thus, whereas tiller formation is somewhat inhibited by straw cover, the slower rate of growth of those which are formed probably lengthens the period of vulnerability of plants to attack. This suggests that *T. contraversa* might establish itself in wheat during the winter months if the environment is favorable for growth of the pathogen. Actually, this is possible: infection of winter wheat by *T. contraversa* has been obtained in Germany following inoculation in November, December, January, and even as late as mid-March (2, 3, 15). Results in Table 3 indicate that, in this experiment, some critical stage in the development of dwarf bunt was in progress during January and February, and that coverings of straw helped to provide an environment favorable for the infective activities of the pathogen. The highest percentages of diseased tillers occurred in plots covered with straw from December 1 to February 25. On the other hand, dwarf bunt was depressed somewhat by removing the straw on January 4 or allowing it to remain on the plots until April 19. The reasons for such results are not apparent, but examination of climatological records suggests that temperature in the micro-environment of the plants could have been one of the critical factors affected by straw cover.

Reports from widely separated areas seem to indicate that high soil moisture and cool soil temperatures favor development of dwarf bunt in winter wheat. In the United States in the Pacific Northwest, however, dwarf bunt has been observed in both the irrigated and dry-farming areas, and it is believed that the pathogen is adapted to a variety of soil types and soil-moisture levels (9). In Germany, Warmbrunn (19) noted that dwarf bunt was most prevalent at higher elevations, where cool temperatures prevailed and there was heavy precipitation. Niemann (14), in Germany, obtained the highest percentages of dwarf bunt at temperatures of 0–10°C and soil-moisture levels of 60 per cent of saturation.

Data accumulated from plots not covered with straw during the winter wheat seasons of 1953 to 1957 show that there were about twice as many dwarf-bunted tillers in 1954–55 and 1955–56 as in 1953–54

and 1956–57. Records for the 3-month period October–December for the area show totals of 10.0 and 11.5 in. of rainfall, respectively, for the first pair of seasons, but only 5.0 and 6.5 in., respectively, for the second pair of seasons. Winter snow cover was heavier and more persistent in the seasons when dwarf bunt was more prevalent than it was in the seasons of lower prevalence. Though not conclusive, the relationship just described seems to emphasize the importance of soil moisture in relation to dwarf bunt.

Results from certain experimental plantings in the dwarf bunt nursery at Ithaca also suggest a close relationship of soil moisture to development of the disease. Inspection of "basin" and "regular" plantings at intervals during autumn and early winter sometimes revealed the presence of standing water in the "basin" plantings. The prevalence of tillers affected by dwarf bunt was 30–40 per cent higher in "basin" than in "regular" plantings (Table 2). It appears that excessive surface water, which at times accumulated in the low-lying poorly draining "basin" plots, in some way favored the activities of the pathogen and/or predisposed the plants to attack. The increased incidences of dwarf bunt in wheat on compacted soils (4, 9) might have been induced partly by high soil moisture resulting from slow drainage and accumulation of excess water in surface depressions formed during compaction of the soil. Compaction of soil as described herein, however, did not influence the development of dwarf bunt (Table 3).

Shallow planting of wheat in infested soil favors development of dwarf bunt (12, 13). Moreover, inoculum on the ground surface (1, 2, 3, 15) or mixed with soil at the surface (Table 3) has high disease-producing potential. In Germany, dwarf bunt was induced by applying inoculum on plots of established plants during autumn and winter months (2, 3, 15). In New York, Dewey (4) inoculated plants ranging from 2 to 21 days old and obtained infection in some plants of all age groups; the results from Germany show, however, that older plants can be attacked. Probably, as Dewey suggested, the pathogen may gain ingress into older plants through young, later-formed tillers if weather factors are favorable for its activities. It is likely, therefore, that coverings of straw or snow help protect the more or less exposed inoculum from unfavorable extremes of weather and provide an environment favorable for attack over a protracted period.

Observations in the Pacific Northwest up to 1949 led to the suggestion that *T. contraversa* may persist in the soil "at least 4 and possibly as long as 7 years" (9). Since then, however, the fungus has been found on species in 8 genera of the Gramineae (5, 6, 8). Plants of susceptible species might therefore tend to perpetuate the fungus in the soil in the absence of wheat, complicating the problem of determining its true ability to survive in the soil. It was reported in 1953, however, that some spores of *T. contraversa* survived 5 years in an outdoor environment on the soil (17). Recent attempts to germinate spores that have weathered outdoors on the ground since August, 1947, reveal that some can survive at least 10 years under the conditions imposed on them.[3] Nevertheless it appears probable that the viability of much of the soil-harbored inoculum diminishes rapidly. The latter is supported by data in Table 1, concerning the incidences of dwarf bunt caused by 1954-collected inoculum. The only difference between the infested batches of soil labeled "1954 weathered" and "1954 not weathered" was the intermittent wetting of one batch by rain; soil moisture appears to have been the factor responsible for lowering the disease-producing potential of the exposed infested soil while it was in storage. Water is essential in the process of spore germination, and a dry soil would not provide an environment favorable for germination of *T. contraversa* spores. Thus, the disease-producing potential of dry infested soil is maintained while that of moist infested soil is reduced: the wetted spores germinate and exhaust themselves. Moreover, moist soils tend to encourage the active processes of microflora in the soil, and there is some evidence that soil microflora may stimulate spores of *T. contraversa* to germinate (7). Reduction of the load of viable spores harbored by the soil would tend to depress the disease-producing potential of infested soil; this is clearly indicated by certain data in Tables 1 and 2, which show that the incidences of dwarf bunt in winter wheat were related to the load of inoculum in the soil.

Results given in this paper, and some of those reported by others working in widely separated regions, point to a relationship between climatological factors and the development of dwarf bunt. The influence of such variable factors as precipitation, snow cover, temperature, light, etc., together with farming practices that are considered highly important by Holton, Kendrick, and Meiners (10), may control the more or less cyclic rise and fall of dwarf bunt.

Department of Plant Pathology
Cornell University
Ithaca, New York

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SOIL FUMIGATION EXPERIMENTS FOR THE CONTROL OF THE SOYBEAN CYST NEMATODE, HETERODERA GLYCINES[1]

B. Y. Endo and J. N. Sasser[2]

SUMMARY

Several soil fumigants were evaluated for control of the soybean cyst nematode, *Heterodera glycines* Ichinohe. Treating infested soil with D-D at 40 and 60 gal./A., Telone at 40 gal./A., Nemagon at 5 gal./A., and methyl bromide at 1, 2, and 3 lbs./100 sq. ft increased plant growth and soybean yield. Root nodulation in infested soil was increased by treatment with nematocides. Although nematode populations were greatly reduced by fumigation, these differences were no longer significant at harvest time, indicating a rapid build-up of the nematode during the growing season.

The soybean cyst nematode, *Heterodera glycines* Ichinohe 1952, was reported in southeastern North Carolina and the United States in 1955 (9). It was previously reported in Japan, Korea, and Manchuria (3), where it causes a severe disease of soybean (*Glycine max* (L.) Merr.) known as "yellow dwarf." Since its initial discovery in North Carolina, it has been found in Tennessee, Missouri, Arkansas, Kentucky, and Mississippi. The disease is very destructive in restricted areas in North Carolina.

The parasitism, morphology, and ecology of the soybean cyst nematode in Japan have been studied by Ichinohe (3, 4). Hirschmann (2) made comparative morphological studies of the soybean cyst nematode in North Carolina and the clover cyst nematode, *H. trifolii* Goffart 1932, a closely related species also occurring in that state. Various biological aspects, including host range, life cycle, and effects of desiccation on cyst contents, were reported by Skotland (7). Chemical treatments of bulbs and corms that harbor the cyst nematode as contaminants were reported by Winstead and Skotland (8). The primary purpose of this investigation was to study the effectiveness of several soil fumigants on control of the nematode in the field. A preliminary report has been given (1).

MATERIALS AND METHODS.—Dowfume W-85 (83% 1,2-dibromoethane), D-D (1,3-dichloropropene; 1,2-dichloropropane), Telone (95% 1,3-dichloropropenes), Nemagon (1,2-dibromo-3-chloropropane), and methyl bromide were applied to soil heavily infested with the soybean cyst nematode about 1 month before soybeans were planted. Rates used were: Dowfume W-85, 4½, 9, and 13½ gal./A.; D-D, 20, 40, and 60 gal./A.; Telone, 20 and 40 gal./A.; Nemagon, 3 and 5 gal./A.; methyl bromide, 1, 2, and 3 lb./100 sq. ft. Dowfume W-85, D-D, and Telone were applied with a MacLean Fumigun. The soil of the plots was first cross-marked to a 12-in. grid, and injections, at a 6-in. depth, were made at the intersections and half-way between intersections in alternate rows of the grid. Granular Nemagon (10%) active ingredient was dis-

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[2] Nematologist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and Associate Professor, Department of Plant Pathology (Nematode Diseases), North Carolina State College, Raleigh, respectively.

tributed over the surface of the soil and mixed with the upper 6 in. of soil with a roto-tiller. Methyl bromide gas was applied under a polyethylene cover. Each treatment was replicated 3 times, and untreated plots were included as checks. The experimental design was a randomized block with plots 20 x 20 ft., each separated by a 4-ft. alleyway seeded to fescue. Eight rows of soybeans (Lee variety) were planted in each plot 1 month after fumigation.

The initial field tests, made in 1956, were repeated in 1957 with the same plots, materials, and rates, except that methyl bromide was excluded.

Soybean cyst nematode populations in the soil in 1956 were determined for each plot before fumigation (April 21) and on the following dates: May 28, June 13, August 14, and August 28. Nematode populations were also determined on December 18, and in 1957 on July 31 and October 31. Population studies were based on larval emergence from cysts isolated from the soil. Soil samples were taken with a sampling tube to a depth of 6 in. from 9 random locations in each plot on each sampling date. The composite sample was mixed in the laboratory, and a ½-pint subsample, for the population study, was thoroughly mixed in a 10-qt. pail ⅔ full of water. The mixture was poured through a 20-mesh sieve nested in an 80-mesh sieve. Cysts and debris collected on the 80-mesh sieve were placed on a single layer of Scotties tissue supported by an aluminum screen in a Baermann funnel containing enough water to cover the cysts. After 10 days the nematodes that had emerged from the cysts were drawn off and counted.

One month after the soybeans were sown, a "white cyst"[3] index was obtained by digging 10 plants at random from the outer 4 rows of each plot and determining the number of white cysts attached to the roots (Fig. 1B). A "white cyst" index was computed from the number of plants in each of the following classes: 0 = no infection; 1 = trace; 2 = light; 3 = moderate; 4 = moderate-heavy; 5 = severe infection.

A "nodulation" index was computed for the above plants from the following class values: 0 = no nodulation (Fig. 1A, right); 1 = slight; 2 = moderate; 3 = heavy (Fig. 1A, left).

Bean yield was obtained for the center 4 rows of each plot at harvest.

"White cyst" index, nodulation index, and yield data were recorded for the tests in both years.

EXPERIMENTAL RESULTS.—Data in Table 1 indicate the numbers of larvae obtained at different dates of sampling. Before fumigation there were no differences between the plots in populations. No viable larvae were recovered from methyl-bromide-treated plots 25 days after fumigation. Nematodes were found in these plots in later samplings, but fewer than in untreated plots. Dowfume W-85 was ineffective in reducing nematode numbers at all sampling dates after fumigation. D-D and Telone varied in effectiveness with the rates used. No viable larvae were recovered for D-D-treated plots 25 days after fumigation at the 40 and 60 gal./A. rates, and only 2 larvae at the 20 gal./A. rate. Later in the season, nematodes were found in all D-D-treated plots, with the lowest numbers at 60 gal./A. Nemagon at 3 gal./A. appeared ineffective, whereas at 5 gal./A. nematode numbers were reduced initially and remained low throughout the growing period. Similarly, Telone at 20 gal./A. was ineffective although Telone at 40 gal./A. resulted in no viable larvae 25 days after fumigation. Later in the season, nematodes were present in plots treated with Telone at 40 gal./A., but fewer than in plots treated at 20 gal./A. At the end of the growing season population levels remained low, but not significantly lower than in untreated plots.

Methyl bromide at 3 lb./100 sq. ft. caused considerable stunting of soybean plants in the center of the plots. There was no evidence of fumigant toxicity from the other treatments.

The "white cyst" index was greatly reduced by many of the treatments (Table 2). All treatments had

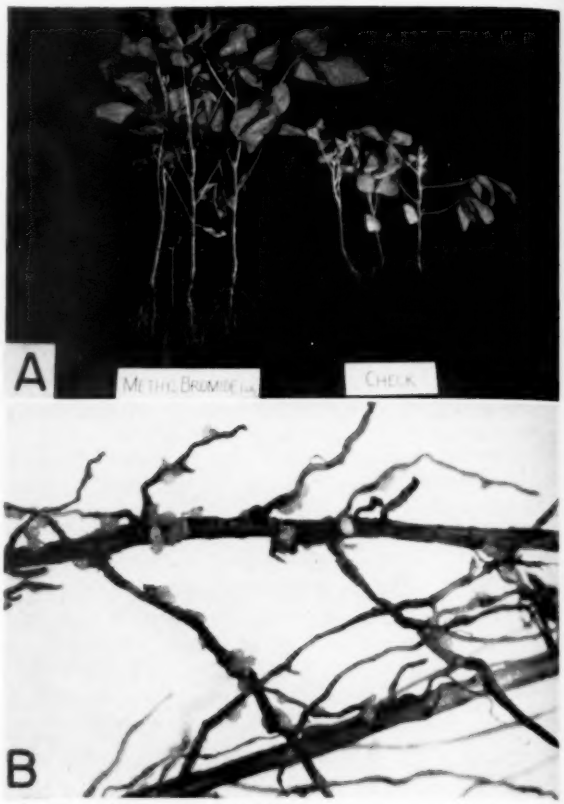

FIG. 1. The effect of fumigation on nodulation and nematode control 1 month after planting. A) Left: soybean plants from methyl-bromide-treated soil; note abundant root nodulation. Right: severely infected soybean plants from untreated control; note absence of nitrogen nodules. B) Highly magnified section of nematode-infected soybean roots showing white cysts and attached egg masses.

[3] "White cyst" as used in this paper means the living adult female; not the true cyst, which is formed from the body of the dead female.

smaller indices than the control, except Telone at 20 gal./A. and Dowfume W-85 at 13.5 gal./A. Similar low indices were obtained with methyl bromide at 1, 2, and 3 lb./100 sq. ft., Telone at 40 gal./A., Nemagon at 5 gal./A., and D-D at 40 and 60 gal./A.

Treatments that altered nematode populations and "white cyst" index also altered root nodulation (Table 2). Nodulation was inversely correlated with "white cyst" index.

Nematocide treatments that were effective also increased the yield of threshed beans (Table 2). Yields for all treatments were better than in the untreated check, except for Telone at 20 gal./A., and Dowfume W-85 at 4.5 and 13.5 gal./A.

The effect of *H. glycines* on soybean plant growth was noted early in the growing season. Soybeans growing in untreated plots and in plots where the fumigant was not effective were noticeably stunted and chlorotic within 2 weeks of emergence, in contrast with plants in plots where nematode populations were reduced. The differences became more pronounced later in the growing season. Fig. 2 shows growth difference, 6 weeks after planting, between a control plot and a plot treated with Nemagon at 5 gal./A.

Before fumigation in 1957, nematode populations in the soil were similar for all plots (according to soil samples taken on December 18, 1956). On July 31 (about 2½ months after fumigation), there were population differences between untreated plots and all fumigant treatments. By October 31, 1957, however, nematode populations were again similar in all the treated plots.

The 1957 "white cyst" index was lower for all

TABLE 1.—*Average larval emergence from cysts isolated from one-half pint of soil collected from each treatment plot both before fumigation and during the growing season, 1956*

Fumigants	Gal./Acre	Soil sampling dates: April 21[a]	May 16	June 13	Aug. 2	Aug. 28
D-D	20	193	2	7	833	3,539
D-D	40	74	2	0	0	1,025
D-D	60	231	0	1	36	468
Dowfume W-85	4.5	42	25	76	101	4,543
Dowfume W-85	9.0	74	9	115	374	2,248
Dowfume W-85	13.5	42	33	86	535	2,910
Nemagon	3.0	79	24	7	230	2,620
Nemagon	5.0	28	7	3	89	543
Telone	20.0	174	44	83	975	2455
Telone	40.0	47	0	3	2	352
Methyl bromide	1 lb.[c]	37	0	0	0	80
Methyl bromide	2 lb.[c]	53	0	9	216	175
Methyl bromide	3 lb.[c]	103	0	0	0	1
Control	None	89	40	218	505	1,249
LSD 1%		NS[b]	38.8	NS[b]	NS[b]	1948
5%		↓	28.7	↓	↓	1440

[a] Sampled before fumigation.
[b] Non-significant when based on analysis of variance. However, significant differences were found when the analysis was based on data transformed to logarithms.
[c] Per 100 sq. ft.

TABLE 2.—*Mean "white cyst" index[a] and nodule index[b] for each treatment about 1 month after planting, and mean yield[c] of soybeans, 1956*

Fumigant	Gal./acre	White cyst	Nodulation	Yield (g)
D-D	20	2.80	2.87	2281
D-D	40	0.07	2.93	2291
D-D	60	0.00	3.00	2423
Dowfume W-85	4.5	1.67	2.00	1299
Dowfume W-85	9	2.33	1.80	2378
Dowfume W-85	13.5	4.00	1.73	1453
Nemagon	3	1.13	2.67	1729
Nemagon	5	0.40	3.00	2243
Telone	5	4.93	.93	1327
Telone	40	0.40	2.67	2554
Methyl bromide	1 lb.[d]	0.0	2.73	2609
Methyl bromide	2 lb.[d]	0.0	3.00	2318
Methyl bromide	3 lb.[d]	0.0	2.27	1733
Control	None	4.67	1.60	738
LSD 1%		1.29	1.03	1159
5%		0.95	0.76	857

[a] Mean "white cyst" index based on 3 replicates of 10 plants each. Individual plants were rated: 0 = no infection; 1 = trace; 2 = light; 3 = moderate; 4 = moderate-heavy; and 5 = severe infection.
[b] Mean nodulation index based on 3 replicates of 10 plants each. Individual plants were rated: 0 = no nodulations; 1 = slight; 2 = moderate; 3 = heavy nodulation.
[c] Mean of 3 replicates harvested from 200 sq. ft. in each treatment plot.
[d] Per 100 sq. ft.

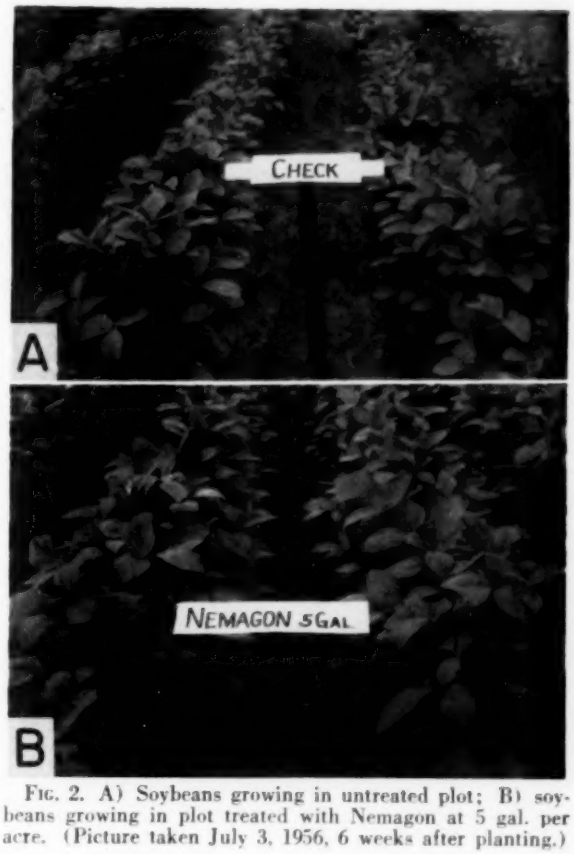

FIG. 2. A) Soybeans growing in untreated plot; B) soybeans growing in plot treated with Nemagon at 5 gal. per acre. (Picture taken July 3, 1956, 6 weeks after planting.)

fumigant treatments, except D-D at the 20 gal./A. rate, than for the untreated plots. Degree of control as measured by "white cyst" index was similar to that in 1956.

In 1957, nitrogen nodulation of the roots was significantly lower for all treatments, except D-D at the 20 gal./A. rate and EDB at 4.5 gal./A. rate, than for the untreated control.

Fumigation treatments again resulted in greater bean yields.

DISCUSSION AND CONCLUSIONS.—The striking increase in growth and yield of soybeans in fumigated soil indicates the destructive potential of the soybean cyst nematode. In control plots and in those plots where the fumigants were ineffective, plant stunting and chlorosis appeared early, and affected plants made poor growth throughout the season. In general, high nematode populations—as measured by soil sample assays during the growing season and by the "white cyst" index 1 month after planting—were associated with poor plant growth and color. Population differences between treated and untreated plots became reversed toward the latter part of the growing season. Where nematode populations had been greatly reduced early in the season, late-season levels were higher than in untreated plots. Apparently, plants severely attacked early in the growing season failed to develop root systems adequate to support rapid nematode multiplication, whereas treated plants provided abundant food throughout the growing season for reproduction by nematodes that had survived fumigation.

The lower yield of beans in plots treated with methyl bromide at 3 lb./100 sq. ft. was undoubtedly due to fumigant injury.

Poor nodulation on plants severely infected with the nematode was reported previously by Ichinohe and Asai (5), but the factors responsible are not understood. It may be that massive larval invasion of young root systems early in the growing season interferes with the invasion of root hairs and the establishment of nitrogen-fixing bacteria.

These tests indicate that a substantial increase in growth and yield can be obtained with the higher rates of the fumigants tested, except for Dowfume W-85. Experiments on the control of another cyst form, *Heterodera rostochiensis* (6), have indicated that ethylene dibromide formulations are not effective against this pest.

Eradication of the soybean cyst nematode in the field appears improbable with the fumigants used in these studies. A combination of fumigation and rotation may keep the nematode under control and greatly reduce the chances of its becoming widely spread.

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"DEEP PLOWING" FOR CONTROLLING VERTICILLIUM WILT OF MINT IN MUCK SOILS[1]

Ralph J. Green, Jr.[2]

SUMMARY

Studies of the vertical distribution and inoculum potential of *Verticillium albo-atrum* var. *menthae* revealed that, in muck soils, this pathogen was absent below 12–18 in., or present only in very low concentration. A soil inversion technique was developed whereby the upper 12–14 in. of topsoil was dropped into the previous plow furrow before the remainder of the soil was turned to a depth of 28–32 in. Wilt incidence the first year after plowing was reduced from 57.2% to 4.0%, and the second year from 85% to 10.2%. Weed control, soil moisture retention, and resistance to wind erosion were also benefited.

INTRODUCTION.—Peppermint (*Mentha piperita* L.) and spearmint (*M. spicata* L. and *M. cardiaca* L.) in the midwest are grown exclusively on the muck soils of northern Indiana, Michigan, and southern Wisconsin. Production of these crops for their essential oil requires considerable investment in labor, machinery, and equipment. Planting and much of the weeding are done by hand, and recovering the oil requires special distillation equipment.

Production of peppermint and spearmint in most of the older mint-producing areas of the midwest is limited by Verticillium wilt, incited by *Verticillium albo-atrum* Reinke & Berth. var. *menthae* Nelson.[3] Growers in affected areas are now withdrawing heavily infested fields from production and seeking uninfested acreage. But, since the mints are propagated vegetatively, the pathogen has spread rapidly on propagative rhizomes with the migration of the industry.

Progress toward control through genetic resistance of the host or through the use of chemical soil amendments has been slow. Although highly resistant mints exist, the problems of oil quality and, in particular, the genetic complexity of peppermint make difficult the development of a commercially acceptable hybrid. Until suitable wilt-resistant hybrids or practical control procedures are available, certain cultural practices can reduce the disease in established plantings.

Little is known concerning the survival of *V. albo-atrum* var. *menthae* in muck soils or its vertical distribution in soil strata. Wilhelm,[4] studying various mineral soils in California, found *V. albo-atrum* to depths of 36 in. The heaviest infestation was concentrated in the upper 0–12 in. The different genesis of muck soils warrants a similar study for this soil type.

The studies reported herein concern the vertical distribution of *V. albo-atrum* var. *menthae* in the various strata of muck soils and the application of this information to developing a cultural practice for the control of Verticillium wilt of peppermint and spearmint.

METHODS AND PROCEDURE.—In April, 1955, soil samples were taken on different farms from 6 fields known to be heavily infested with *V. albo-atrum* var. *menthae*. Three samples were taken at random from each field in the following manner. Soil samples were taken at 6-in. intervals from one side of a trench 24 in. wide, 6 ft. long, and 36 in. deep. The samples from each stratum were bulked and thoroughly mixed in the greenhouse, and sufficient soil was taken from each bulk sample to fill 2 metal soil trays, each $30 \times 42 \times 8$ in., equipped with drains. Fifteen cuttings of susceptible peppermint plants were planted in each soil tray.

After 65 days, the test plants were lifted, green weights were recorded, and stem sections were indexed for the pathogen in the laboratory. The peppermint plant produces numerous aerial shoots from stolons growing on or below the soil surface. A shoot with an independent root system was considered a separate plant. Stem sections from each plant were surface-sterilized for 5 minutes in 1% sodium hypochlorite (Clorox) and plated directly to modified Czapek's agar. Readings were made after 7–10 days of incubation.

Since the pathogen proved absent below 12–18 in., or present only at very low intensities, and since the subsoil supported vigorous plant growth, it appeared feasible to develop a plowing technique whereby the heavily infested topsoils could be replaced with the wilt-free subsoils. Although muck soils vary significantly in depth, type of subsoil, etc., the typical muck-type soil of the Midwest has a friable, black, partially oxidized topsoil 10–16 in. deep and a subsoil of virtually undecomposed peats that may contain deposits of alluvial clays, sand, crustacean deposits (marl), etc. Thus, the soil stratum at 12–18 in. marks the

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[2] Assistant Professor of Plant Pathology, Purdue University, and Agent, Crops Research Division, Agriculture Research Service, U. S. Department of Agriculture.

[3] Nelson, R. 1950. Verticillium wilt of peppermint. Mich. State Univ. Agr. Expt. Sta. Tech. Bull. No. 221.

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lower limits of the oxidized portions of the soil profile. These soils are light weight and free of obstructions other than plant debris.

A "deep plowing," or soil-inversion, technique was developed in cooperation with a grower to take advantage of the limited vertical distribution of the wilt pathogen in muck soil. A frame-mounted 36-in. moldboard plow was modified by adding a 16-in. jointer plow to the frame immediately ahead of the large moldboard (Fig. 1). The small plow drops the upper 12–14 in. of topsoil into the previous furrow before the remainder of the subsoil is turned by the 36-in. plow. A large coulter is usually attached to the forward part of the plow tongue to cut the furrow cleanly. Since muck soils are free of obstacles, the plow depth can be accurately controlled at 28–32 in. The modified plow unit is readily drawn by a Caterpillar tractor, model TD-9, and speeds of 2½–3 mph are required to turn the subsoil completely. An experienced operator can plow 6–10 acres per day. Plowing can be done during the late fall and winter, when labor and machinery demands are less acute.

In October, 1955, plots were selected at the Purdue University Muck Crops Farm to compare the incidence of Verticillium wilt in a "deep plowed" test area and an area plowed with conventional equipment. Two adjacent areas 100 × 750 ft. were plowed —one 28–30 in. deep and the other 6–8 in. The "deep plowed" area was plowed in 1 direction only. No attempt was made to manage the 2 plot areas separately during disking and seed-bed preparation.

A 5-year planting sequence was established, using peppermint, corn, and potatoes, with each treatment replicated 4 times. At the end of the 5-year period the entire area would be planted to peppermint. The plots, at right angles to the direction of plowing, were continuous across the conventional and "deep plowed" areas. The peppermint plots were planted with disease-free rhizomes and maintained in rows the first year. These plots were plowed down to 4–6 in. in the fall to prevent winter injury, and came up the second and subsequent years as meadow mint.

TABLE 1.—*Vertical distribution of V. albo-atrum var. menthae in muck soils*

Sample	No. of plants	Top wt. (g)	No. infected	% infected
0–6 in	36	966	35	97.2
6–12 in.	40	908	36	90.0
12–18 in.	40	1060	21	52.5
18–24 in.	41	1589	0	0.0
24–30 in.	40	1324	0	0.0
30–36 in.	44	806	2	4.5

Disease incidence was determined by plant counts in the plots of row mint and by counts and visual estimations in the second-year meadow mint plots.

RESULTS.—The vertical distribution and inoculum potential of *V. albo-atrum* var. *menthae* in muck soils exhibited considerable variation in the soil strata sampled (Table 1). A sharp decline in infection incidence below 12–18 in. corresponds to the lower limits of the partially oxidized topsoil of most of the soils sampled.

It will be noted that, except for the 30–36 in. stratum, the non-oxidized subsoils supported vigorous plant growth. The relatively poor growth in this sample was due to extremely low fertility, and was corrected by the addition of suitable nutrients. Reduction in total growth (top wt.) of plants grown in the 0–6 in. and 6–12 in. increment samples was the result of plant mortality before harvesting for indexing. This experiment was repeated several times with similar results. Infection incidence below 12–18 in. was 0–1.5%. Since some mixing of soil samples un-

FIG. 1. Frame-mounted 36-in. moldboard plow (A), with attached jointer plow (B), used to "deep plow" muck soils for the control of Verticillium wilt of peppermint and spearmint.

TABLE 2.—*Wilt incidence and yield compared in soils plowed at 6–8 in. and 28–30 in. (2 years)*

Plow depth	Row mint '56	Row mint '57		Meadow mint '57[a]	
	% Infection	% Infection	Oil yield	% Infection	Oil yield
			lb./A.		lb./A.
6–8 in.	57.2	72.6	12.2	85.0	26.5[b]
28–30 in.	4.0	8.6	24.2	10.2	38.9

[a] Second-year crop.
[b] LSD at 5%: 5.4 lb./A.

doubtedly occurred, this may account, in part, for the infection incidence in the subsoil increments.

The data presented in Table 2 represent the levels of disease incidence and oil yields from adjacent "deep plowed" and conventionally plowed areas 2 years after treatments. The reduction in disease incidence, from a mean of 57.2% on soils plowed 6–8 in. deep to 4.0% in "deep plowed" plots, was accompanied by a corresponding increase in oil yields. In 1957, disease incidence was somewhat higher in all plots. Most of the increase on "deep plowed" soils was due to encroachment from adjacent areas, apparently the result of soil transport during planting, weeding, and cultivation. No attempt had been made to manage the 2 test areas separately, since it was considered desirable to know whether reinfestation would occur, the rate, and whether the inoculum level would exceed the level before "deep plowing."

DISCUSSION AND CONCLUSIONS.—The soil inversion technique, "deep plowing," has proven practical for the control of Verticillium wilt of peppermint and spearmint on muck soils. The additional costs for equipment and labor have been offset by increased yields and certain other cultural advantages, such as weed control, improved moisture retention in the topsoil, and reduced hazard of wind damage in the spring. Other crops, such as onions, potatoes, and corn, are successfully grown on these soils where fertility levels are re-established and certain trace-element deficiencies corrected.

There are certain disadvantages associated with "deep plowing" that may discourage this practice. Since the muck soils of the Midwest vary in depth and subsoil characteristics, some areas may be too shallow or heterogeneous for plowing at depths of 28–32 in. (Careful sampling of the field with a soil probe, to determine depth of the organic layer and nature of the subsoil, is advantageous.) Other disadvantages include increased frost hazard and deficiencies of certain trace elements, such as copper, zinc, and manganese.

It is not known how long soils plowed to depths of 28–32 in. will remain relatively disease-free. It has been demonstrated that the pathogen will eventually reinfest these soils by encroachment. Thus, it seems probable that the absence of the pathogen from the subsoils initially is a physical rather than a biological phenomenon. There is no evidence that the pathogen will not colonize the peat-like subsoils or that it is inhibited, biologically, by the other soil microflora or fauna.

Currently, more than 10,000 acres have been "deep plowed," using the equipment and techniques described herein. The practice is effective only if the heavily infested topsoil is first dropped into the previous furrow before the remainder of the soil is turned. Incomplete inversion of the soil leaves remnants of the topsoil within the root zone of the susceptible mint plants.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY
PURDUE UNIVERSITY
LAFAYETTE, INDIANA

BOOK LISTINGS

Forestry Terminology. 1958. Revised 3rd edition. 97p. Published by the Society of American Foresters, Mills Building, Washington 6, D. C. (Monumental Printing Co., Baltimore, Maryland). Price $3.50

This volume contains concise definitions of all terms commonly found in writings on forestry. These definitions are simple and easily understood. Only rarely is a definition too brief or too technical to convey the meaning adequately to non-foresters. In addition to the standard forestry terminology, such fields as genetics, soils, hydrology, meteorology, entomology, pathology, statistics, and wood preservation are given elementary coverage. The preface contains an up-to-date list of similar glossaries for allied sciences. Plant pathologists who follow the forest disease literature, or who have an active interest in any of the facets of forestry will find this book a valuable reference.

ERDTMAN, G. 1958. **Pollen and spore morphology/Plant taxonomy.** Volume II. Almquist and Wicksells, Stockholm, Sweden. Distributed by the Ronald Press Co. New York. 150 p. Price $8.00 (In English).

Volume II of a 3 volume series, is subtitled *An Introduction to Palynology* and deals with pollen and spore morphology in the Gymnosperms, Pteridophytes, and Bryophytes. The first section consists entirely of illustrations with captions. The later volume will provide the text for these illustrations. A supplement contains palynological methods and techniques. One by B. M. Afzelius is entitled *On New Methods in Physical Cell Research and their Application in Studies of Pollen Grains and Spores;* the other by J. Radwan Praglowski is entitled *On the Cutting of Ultra-thin Sections.*

Host-parasite Relationship of the Soybean Cyst Nematode in Resistant Soybean Roots. J. P. Ross.[1] The histopathology of roots of resistant soybean (*Glycine max* L.) infected by *Heterodera glycines* Ichinohe is important in understanding the nature of resistance. Although studies have been conducted on the response of susceptible plants to cyst nematodes, little information was found in the literature on the histopathological aspects of cyst nematode infections in roots of resistant plants. Franklin[2] summarized the status of Heterodera host-parasite relationships up to 1949. Ichinohe[3] gave a brief description of the response of soybean root tissue to the feeding of the soybean cyst nematode. Although Doncaster[4] did not describe in detail the pathological histology of roots of *Solanum nigrum* L. invaded by larvae of *Heterodera rostochiensis* Wollenweber, he nevertheless observed necrosis of invaded roots and proposed that this led to death of the nematode. The purpose of this investigation was to compare the nature of the reaction of roots of resistant and susceptible soybean plants after invasion by infective second stage larvae of *H. glycines.*

The soybean variety Peking, found by Ross and Brim[5] to be resistant to the soybean cyst nematode, and a susceptible line were used as test plants. Seed were planted in field-infested soil in 4-in. pots and grown in the greenhouse. The average daily minimum and maximum temperatures during the growing period were about 21 and 38°C. Soil was washed from the roots of 1 pot each of both susceptible and resistant plants 10, 14, 20, 27, and 32 days after planting. Roots were stained for 2 minutes in boiling lactophenol-acid fuchsin, washed briefly in tap water, and cleared in lactophenol as described by McBeth et al.[6]

[1] Plant Pathologist, U. S. Department of Agriculture, Agricultural Research Service, Crops Research Division; and North Carolina Agricultural Experiment Station.

[2] Franklin, Mary T. 1951. The cyst-forming species of Heterodera. Wilding and Son, Shrewsbury, England. 147 p.

[3] Ichinohe, M. 1955. Studies on the morphology and ecology of the soybean nematode, Heterodera glycines, in Japan. Rept. Hokkaido Natl. Agr. Expt. Sta. 48: 1-64. (In Japanese with English summary).

[4] Doncaster, C. C. 1953. A study of host-parasite relationships. The potato root eelworm (Heterodera rostochiensis) in black nightshade (Solanum nigrum) and tomato. J. of Helminthol. 28: 1-8.

[5] Ross, J. P., and C. A. Brim. 1957. Resistance of soybeans to the soybean cyst nematode as determined by a double-row method. Plant Disease Reptr. 41: 923-924.

[6] McBeth, C. W., A. L. Taylor, and A. L. Smith. 1941. Note on staining nematodes in root tissue. Proc. Helminthol. Soc. Wash. D. C. 8: 26.

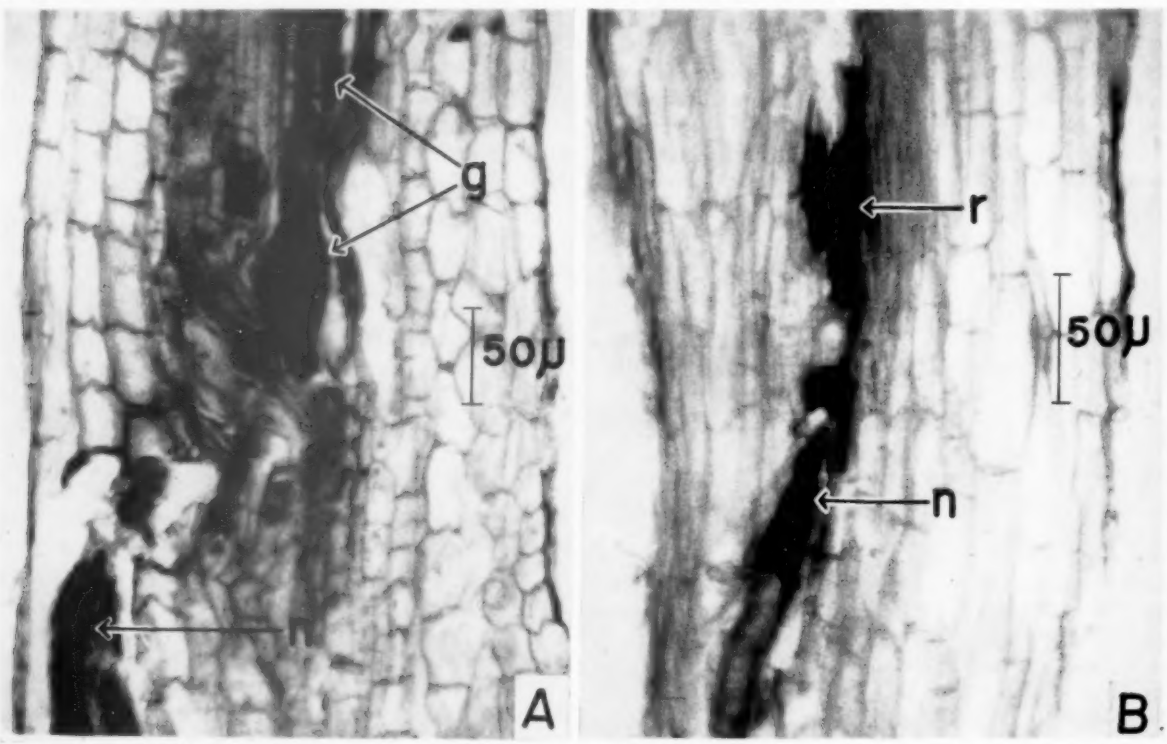

FIG. 1. Longitudinal sections of roots of soybean infected with the soybean cyst nematode. A) Root from a susceptible plant showing giant cell (g) and developing adult male (n). B) Root from a resistant plant showing the necrotic reaction of the host tissue (r) and the adversely affected larva (n).

Sections of roots 0.5 cm in length containing the nematodes were removed, dehydrated by passage through a graded ethanol series, and imbedded in paraffin. Sections were cut 14 μ thick, mounted on slides, and stained with safranin-fast green. During the dehydration process the nematodes were completely destained.

Examinations of the sections revealed that, after entering the root, second-stage larvae migrate through the cortex and severely damage many cells in their path. In roots of both resistant and susceptible plants many cortical cells were broken. The cells around the larvae frequently contained densely red-stained, coarse, granular material. This seemed to occur commonly in resistant roots, and only occasionally in susceptible roots.

Only when larvae became sedentary were sharp differences manifested between resistant and susceptible plants. As commonly reported, the larvae were so oriented that their head region projected into the vascular tissue while the body proper lay in the cortex. In certain sections containing more than 1 feeding larva, it was observed that orientation of the nematodes with respect to the root tip was not always the same.

The first giant cells were found in roots of susceptible plants sacrificed 14 days after planting. These cells stained green and were located in the feeding area of second-stage larvae adjacent to vascular tissue. In roots lifted at subsequent sampling dates, giant cells were commonly found in connection with all stages of larvae and adults (Fig. 1A). These abnormal cells are similar to those described by other authors.[2,3] In some instances considerable disruption and dislocation of vascular elements, resulting from extensive giant cell formation, were observed in the stele.

In roots of resistant plants, no giant cells were observed. The cells about the head region of the nematode, instead of being enlarged and stained green, were stained deep red and appeared disorganized and necrotic (Fig. 1B). No fourth stage or adult females were found in resistant plants, and some third stage larvae appeared to be in a state of degeneration. However, the appearance of fourth stage and adult males in the roots of resistant plants was common. In all cases the cells in feeding areas of all nematodes were necrotic.

The development of adult males, and not of adult females, is interesting and corroborates Ichinohe's statement[5] that the male of *H. glycines* requires less nourishment to mature than the female. Apparently the male is capable of obtaining enough food to complete its life cycle in spite of the necrotic reaction of the cells. It should be pointed out that an occasional female adult has been observed on the roots of Peking plants in the field. It is not known whether this reflects genetic variation in soybean lines or in the nematode, or is due to some other factor.

It is considered that the reaction of this resistant variety to *H. glycines* indicates a type of hypersensitivity. Mizogami[7] reported a similar type of reaction of certain lines of sweetpotato to the root-knot nematode, *Meloidogyne* spp.—Plant Pathology, North Carolina State College, Raleigh, North Carolina.

[7] Mizogami, T. 1947. An anatomical study on the resistance of sweetpotato to root-knot nematode. (In Japanese). Proc. Meeting Agr. Research Workers in Kyushu 1: 19-20.

Germination Studies with Spores of the Dwarf Bunt Fungus. WADE G. DEWEY AND L. J. TYLER.[1] A factor that contributes to the sporadic occurrence of dwarf bunt is the irregularity with which spores of the causal organism, *Tilletia contraversa* Kühn, germinate. Whereas spores of the species that cause common bunt germinate quite readily over a relatively wide range of environmental conditions, spores of the dwarf bunt fungus appear to be much more exacting in their germination requirements. This factor undoubtedly contributes to the survival of *T. contraversa* in the soil over extended periods.

Numerous attempts have been made to stimulate spore germination by regulating factors in the environment. Low temperatures were shown by Holton,[2] Lowther,[3] and others, to be essential for the germination of dwarf bunt spores. An apparent stimulatory effect of light has been demonstrated by a number of investigators.[4,5,6,7] Treatment with various chemicals and the use of soil-extract media have been reported to have a favorable effect on spore germination.[8,9]

Although these and other investigations have yielded treatments by which the germination of

[1] Respectively, Assistant Professor of Agronomy, Utah State University, Logan, Utah; and Professor of Plant Pathology, Cornell University, Ithaca, New York.

[2] Holton, C. S. 1943. Chlamydospore germination in the fungus causing dwarf bunt of wheat. Phytopathology 33: 732-735.

[3] Lowther, C. V. 1948. Low temperature as a factor in the germination of dwarf bunt chlamydospores. Phytopathology 38: 309-310.

[4] Gassner-Hoechst, G. G. 1953. Untersuchungen über Keimungsbedingungen und Bekampfungsmoglichkeiten beim Zwergbrand (Tilletia brevifaciens) im Laboratorium. Phytopath. Z. 21: 53-62.

[5] Gassner, G., and E. Niemann. 1954. Untersuchungen über die Temperatur—und Lichtabhangigkeit der Sporenkeimung verschiedener Tilletia-Arten. Phytopath. Z. 21: 367-394.

[6] Pichler, F. 1953. Zur Frage der Keimung von Roggen- und Zwergsteinbrandsporen. Pflanzenschutz Ber. 11: 12-17.

[7] Baylis, R. J. 1955. Field infection experiments with dwarf bunt of winter wheat in Ontario. Plant Disease Reptr. 39: 159-160.

[8] Gassner, G., and E. Niemann. 1955. Uber die Beeinflussung der Sporenkeimung des Zwergsteinbrandes und Roggensteinbrandes durch verschiedene Chemikalien. Phytopath. Z. 23: 121-140.

[9] Gassner, G., and E. Niemann. 1955. Synergistische und antagonistische Wirkung von Pilzen und Bakterien auf die Sporenkeimung verschiedener Tilletia-Arten. Phytopath. Z. 23: 395-418.

dwarf bunt spores can be influenced, as yet none of them have shortened the germination period below 3–4 weeks.

The studies here reported were an attempt to shorten this period by exposing dwarf bunt spores to certain heat, scarification, and chemical treatments. Treated spores were distributed on Petri plates containing 2% water agar and examined microscopically every 5 days for 2 months. Per cent germination was recorded for each 5-day interval until germination reached a stage where mycelial and pro-mycelial growth made counts inaccurate. Throughout the tests the plates were kept under artificial light at 5–8°C.

Five series of treatments were applied: series I involved 3 growth-regulating compounds; series II involved 3 inorganic acids; series III involved 3 bases; series IV involved scarification with carborundum dust; series V involved heat treatment. In series I the growth regulators 2,4-D, indole-3-acetic acid, and naphthalene acetic acid were added to water agar in concentrations of 1, 10, and 100 ppm. A plate containing 10% orange juice in water agar was included in series I. After the agar had solidified, a suspension of spores in a few ml of distilled water was swished over the surface and the excess liquid removed by inverting the plate.

Series II and III dealt, respectively, with 3 acids (H_2SO_4, HNO_3, and HCl) and 3 bases (KOH, NaOH, and NH_4OH). Each was used at a 10% concentration, and spores were exposed to the chemicals for periods of 1, 5, and 10 minutes. Treated spores were washed 5 times in distilled water before plating.

In series IV a mixture of spores and #600 carborundum powder was ground in a mortar for different periods. Before plating, spores and carborundum were separated by differential centrifugation.

In series V, 25 time-temperature combinations were used. In one set of treatments spores were presoaked according to the procedure followed in the hot-water treatment of seed for the control of loose smut. The spores were soaked 4–6 hours in cold water, exposed 2 minutes to 120°F, and held at 129°F for 1, 5, 8, 10, and 15 minutes. In a second set of treatments, spores were exposed to 129°F for the same intervals without prior soaking. In a third set the spores were held ½, 1, 5, 10, and 15 minutes at 150°F. In a fourth set, dry spores were exposed to 150°F in an oven for ½, 1, 5, 10, and 15 minutes. In the fifth set, spores were held in an oven at 170°F for ½, 1, 5, 10, and 15 minutes. In the first 3 sets of heat treatments the spores were exposed to the various temperatures in a distilled-water suspension. The spores exposed to dry heat were inserted and removed through the exhaust vent of a laboratory oven.

A control plate in each of the 5 series contained a sample of the untreated spores.

None of the treatments shortened the period required for germination under that recorded for the controls. All treatments in the acid- and base-treated series resulted in the death of the spores or severely inhibited their germination. The only acid treatment in which any appreciable germination was noted was one in which spores were exposed 1 minute to 10% H_2SO_4. No more than trace germination was observed in any of the base-treated plates.

Decreased germination was general in the series treated with growth regulators, except for the treatments with 1 and 10 ppm indole-3-acetic acid. In these, germination approximated that of the control. No germination was obtained on the 10% orange juice medium.

The scarification treatments with carborundum dust were included to test the possibility that the characteristic hyaline sheath surrounding dwarf bunt spores might serve to inhibit germination by reducing water uptake or by retaining inhibitory substances within the spore. Microscopic examination of treated spores revealed a large number of loose sheath fragments and many spores lacking part or all of their sheath. The fact that germination following scarification did not significantly vary from that of untreated spores seems to indicate either that the sheath constitutes no real deterrent to germination or that its removal by this means resulted in death or impairment of the spore.

The various heat treatments were designed to test temperature shock as a possible means of inducing germination of dwarf bunt spores. Although no stimulatory response was noted, it was found that the spores in a dry condition were able to withstand short exposures to rather high temperatures. The most severe time-temperature combination used, 15 minutes at 170°F, apparently did not sample the upper limit, inasmuch as considerable germination was observed in spores receiving this treatment. However, germination fell off rapidly when spores were exposed to high temperature in an aqueous suspension. It is interesting to note that the hot-water treatment recommended for control of loose smut of wheat only slightly retarded the germination of dwarf bunt spores.—Departments of Plant Breeding and Plant Pathology, Cornell University, Ithaca, New York.